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Review

Polyhydroxylated alkaloids — natural occurrence and therapeutic applications

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Abstract

Over one hundred polyhydroxylated alkaloids have been isolated from plants and micro-organisms. These alkaloids can be potent and highly selective glycosidase inhibitors and are arousing great interest as tools to study cellular recognition and as potential therapeutic agents. However, only three of the natural products so far have been widely studied for therapeutic potential due largely to the limited commercial availability of the other compounds.

Keywords: Glycosidase; Inhibitors; Pyrrolidines; Piperidines; Pyrrolizidines; Indolizidines; *Nor*tropanes; N-Containing sugars

Contents

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1. Introduction

Drug discovery programmes have typically used solvents such as methanol, chloroform or hexane for extraction. However, most preparations used in traditional medicines are formulated in water and carbohydrates and glycosides which are sparingly soluble in dry methanol and less polar solvents are important biomolecules. It is becoming apparent that the water soluble fractions of medicinal plants and microbial cultures contain many interesting novel structures including carbohydrate analogues. Such analogues include a rapidly growing number of reported polyhydroxylated alkaloids with molecular weights below 250 Da which show great stereoselectivity in biological activity. Such selectivity is not surprising when the chemical and biological diversity in terms of structural information of small sugars is considered. For instance, there are 2^9 stereoisomers of sucrose alone and even glucose has 2×2^5 possible isomers in the pyranose and furanose forms. This amazing diversity in such small molecules displays a remarkable economy in structural information in nature, completely surpassing in molecular weight terms anything achieved by the amino acids. The polyhydroxylated alkaloids found in plants and micro-organisms are arousing considerable interest as potential therapeutic agents and as tools used to understand biological recognition processes and so this paper will review their distribution and therapeutic activities known to date.

The first natural polyhydroxylated alkaloid to be detected was the piperidine alkaloid nojirimycin (Fig. 1a) which was isolated from a *Streptomyces* filtrate in 1966 by Inouye et al., but most have been discovered since 1983. These alkaloids can be considered as analogues of monosaccharides in which the ring oxygen has been replaced by nitrogen. They are monocyclic and bicyclic polyhydroxylated derivatives of the following ring systems: pyrrolidine, piperidine, pyrrolizidine (two fused pyrrolidines with N at the bridgehead), octahydroindolizine or indolizidine (fused piperidine and pyrrolidine) and *nor*tropane. Systematically, these alkaloids have been described in the literature as derivatives of the parent heterocyclic compounds or sugars. 1-Deoxynojirimycin (2*S*-hydroxymethyl-3*R*,4*R*,5*S*-trihydroxy-piperidine or 1,5-dideoxy-1,5-imino-D-glucitol; Fig. 1b) was originally

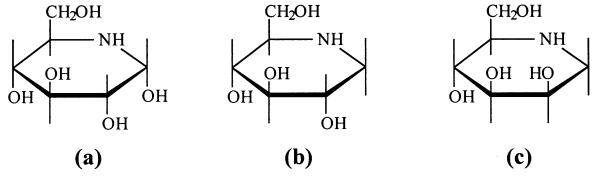

Fig. 1. Diagram illustrating the common nomenclature for polyhydroxylated piperidine alkaloids: (a) 5-amino-5-deoxy-D-glucopyranose has the trivial name nojirimycin; (b) 1-deoxynojirimycin (DNJ) is the trivial name for 1,5-dideoxy-1,5-imino-D-glucitol; and similarly (c) 1-deoxymannojirimycin (DMJ) is the common name for 1,5-dideoxy-1,5-imino-D-mannitol.

synthesised by removing the anomeric hydroxyl group of nojirimycin (Inouye et al., 1968) but it was later isolated from plant sources and bacterial cultures (Yagi et al., 1976; Murao and Miyata, 1980). This derivative is more stable than nojirimycin and has become a model compound in this area of research, giving rise to a trivial nomenclature for 1-deoxy analogues of other alkaloids of this type. Thus, the 1-deoxy piperidine analogue of mannose has been given the trivial name of 1-deoxymannojirimycin (DMJ) (Fig. 1c). The nomenclature used most frequently for particular compounds and their common abbreviations will be used in this review.

2. The distribution of polyhydroxylated alkaloids

Table 1 lists the micro-organisms and plants reported to produce polyhydroxylated alkaloids which have a structural resemblance to carbohydrates. Some of the alkaloids such as 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) and 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1) (Fig. 2) would appear to be fairly widespread secondary metabolites as they have been reported from species of both tropical and temperate plants from quite unrelated families and DMDP is also produced by a species of *Streptomyces* (Watanabe et al., 1995). Others, such as the indolizidine alkaloid castanospermine (Fig. 6), have only been found in two closely related legume genera. The polyhydroxylated pyrrolizidine alkaloids (Fig. 5) appeared to be restricted to the Leguminosae until their discovery in 1994 in a member of the Casuarinaceae and then in the closely related Myrtaceae. Recently they have also been found in abundance in members of the Hyacinthaceae (Kato et al., 1999; Asano et al., 2000a). The polyhydroxylated *nor*tropane alkaloids (Fig. 7) seem to be largely limited to the closely related families Solanaceae and Convolvulaceae, where they co-occur with tropane alkaloids. However, they have also been found in species of *Morus* (Moraceae) (Asano et al., 1994a,b) which is a family not noted for production of tropane alkaloids. [For descriptions of the chemotaxonomic distribution of calystegines in the Solanaceae and Convolulaceae, refer to Griffiths (1998) and Schimming et al. (1998), respectively.]

While there may be phylogenetic reasons for particular distributions of the polyhydroxylated alkaloids in plants, caution should nevertheless be exercised in using the presence of these compounds as taxonomic markers. One reason is that these alkaloids can be released into the soil by producer plants and micro-organisms from whence some such as DMDP and castanospermine can be readily taken up and accumulated in plant tissues of completely unrelated neighbouring species (Nash et al., 1996). It may also be the case that micro-organisms (*Rhizobium*, other rhizosphere organisms, or endophytes) closely associated with specific plants are capable of

Table 1
The naturally-occurring polyhydroxylated alkaloids

Alkaloid	Source and reference
Pyrroline (Fig. 2)	
3,4-Dihydroxy-5-hydroxymethyl-1-pyrroline (Nectrisine or FR-900483)	*Nectria lucida* F-4490 (ATCC 20722) (Ascomycetes) (Shibata et al., 1988)
Pyrrolidines (Figs. 2 and 3)	
2*R*-Hydroxymethyl-3*S*-hydroxypyrrolidine (CYB-3)	*Castanospermum australe* (Leguminosae) seeds/leaves (Nash et al., 1985)
N-Hydroxyethyl-2-hydroxymethyl-3-hydroxypyrrolidine	*Castanospermum australe* (Leguminosae) seeds (Molyneux et al., 1991)
1,4-Dideoxy-1,4-imino-D-arabinitol (D-AB1)	*Angylocalyx* spp. (Leguminosae) seeds/leaves/bark (Jones et al., 1985) *Arachniodes standishii* (Polypodiaceae) leaves (Furukawa et al., 1985; Jones et al., 1985) *Morus bombycis* (Moraceae) leaves (Asano et al., 1994a) *Eugenia* spp. (Myrtaceae) leaves/bark (Nash et al., 1996) *Hyacinthoides non-scripta* (Hyacinthaceae) bulb/leaves (Watson et al., 1997) *Scilla campanulata* (Hyacinthaceae) bulb (Kato et al., 1999) *Adenophora triphylla* var. *japonica* (Campanulaceae) whole plant (Asano et al., 2000b)
1,4-Dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol	*Morus bombycis* (Moraceae) leaves (Asano et al., 1994a]
	Morus alba (Moraceae) roots (Asano et al., 1994b)
1,4-Dideoxy-1,4-imino-D-ribitol	*Morus alba* (Moraceae) roots (Asano et al., 1994b)
2*R*,5*R*-Dihydroxymethyl-3*R*,4*R*-dihydroxy-pyrrolidine (DMDP)	*Derris elliptica* (Leguminosae) leaves (Welter et al., 1976]
	Lonchocarpus spp (Leguminosae) seeds/leaves (Evans et al., 1985a) *Endospermum* sp. (Euphorbiaceae) leaves (Horn et al., 1987) *Omphalea diandra* (Euphorbiaceae) leaves (Kite et al., 1990) *Streptomyces* sp. KSC-5791 (Actinomycetes) (Watanabe et al., 1995) *Nephthytis poissoni* (Araceae) fruit/leaves (Dring et al., 1995; Nash et al., 1996) *Aglaonema* spp (Araceae) leaves (Dring et al., 1995; Asano et al., 1997a) *Hyacinthoides non-scripta* (Hyacinthaceae) bulb/leaves (Watson et al., 1997) *Campanula rotundifolia* (Campanulaceae) leaves (Nash et al., 1997) *Hyacinthus orientalis* (Hyacinthaceae) bulb (Asano et al., 1998a) *Scilla campanulata* (Hyacinthaceae) bulb (Kato et al., 1999) *Adenophora* spp. (Campanulaceae) roots (Ikeda et al., 2000)
6-Deoxy-DMDP	*Angylocalyx* spp. (Leguminosae) seeds/leaves/bark (Molyneux et al., 1993a)
6-*C*-Butyl-DMDP	*Adenophora triphylla* var. *japonica* (Campanulaceae) whole plant (Asano et al., 2000b)
6-Deoxy-6-*C*-(2,5-dihydroxyhexyl)-DMDP	*Hyacinthoides non-scripta* (Hyacinthaceae) fruits/stalks (Kato et al., 1999]
	Scilla campanulata (Hyacinthaceae) bulb (Kato et al., 1999)
2,5-Imino-2,5,6-trideoxy-D-*gulo*-heptitol	*Hyacinthus orientalis* (Hyacinthaceae) bulb (Asano et al., 1998a)
2,5-Dideoxy-2,5-imino-DL-*glycero*-D-*manno*-heptitol (homo-DMDP)	*Hyacinthoides non-scripta* (Hyacinthaceae) (Watson et al., 1997) *Hyacinthus orientalis* (Hyacinthaceae) bulb (Asano et al., 1998a) *Scilla campanulata* (Hyacinthaceae) bulb (Kato et al., 1999) *Muscari armeniacum* (Hyacinthaceae) bulbs (Asano et al., 2000a)

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Table 1 (*continued*)

Alkaloid	Source and reference
Homo-DMDP-7-*O*-apioside	*Hyacinthoides non-scripta* (Hyacinthaceae)) bulb/leaves (Watson et al., 1997) *Scilla campanulata* (Hyacinthaceae) bulb (Kato et al., 1999) *Muscari armeniacum* (Hyacinthaceae) bulbs (Asano et al., 2000a)
Homo-DMDP-7-*O*-β-D-xylopyranoside	*Hyacinthoides non-scripta* (Hyacinthaceae) fruits/stalks (Kato et al., 1999) *Scilla campanulata* (Hyacinthaceae) bulb (Kato et al., 1999) *Muscari armeniacum* (Hyacinthaceae) bulbs (Asano et al., 2000a)
6-Deoxy-homo-DMDP	*Hyacinthus orientalis* (Hyacinthaceae) bulb (Asano et al., 1998a) *Muscari armeniacum* (Hyacinthaceae) bulbs (Asano et al., 2000a)
Gualamycin	*Streptomyces* sp. NK11687 (Tsuchiya et al., 1995)
Broussonetinines A and B, Broussonetines A, B, C, D, E, F, G, H, I, J, K and L	*Broussonetia kazinoki* (Moraceae) branches (Shibano et al., 1997a,b, 1998a,b, 1999a,b)
Piperidines (Fig. 4)	
Nojirimycin	*Streptomyces roseochromogenes* R-468 (Inouye et al., 1966, 1968; Ishida et al., 1967) *Streptomyces lavandulae* SF-425 (Inouye et al., 1966, 1968; Ishida et al., 1967) *Streptomyces nojiriencis* SF-426 (Inouye et al., 1966, 1968; Ishida et al., 1967)
1-Deoxynojirimycin (DNJ)	*Morus* sp. (Moraceae) roots (Yagi et al., 1976) *Bacillus amyloliquefaciens, B. polymyxa, B. subtilis* (Schmidt et al., 1979) *Streptomyces lavandulae* subsp. *trehalostaticus* no. 2882 (Murao and Miyata, 1980) *Omphalea queenslandiae* (Euphorbiaceae) leaves (Kite et al., 1991) *Endospermum medullosum* (Euphorbiaceae) leaves (Kite et al., 1991) *Morus bombycis* (Moraceae) leaves (Asano et al., 1994a) *Hyacinthus orientalis* (Hyacinthaceae) bulb (Asano et al., 1998a) *Adenophora triphylla* var. *japonica* (Campanulaceae) whole plant (Asano et al., 2000b)
1-Deoxynojirimycin-2-*O*-, 3-*O*-, 4-*O*-* α-D-glucopyranosides and 2-*O*-, 6-*O*-α-D-galactopyranoside and 2-*O*-, 3-*O*-, 4-*O*-, 6-*O*-β-D-glucopyranoside	*Morus alba* (Moraceae) roots (Asano et al., 1994b) **Streptomyces lavandulae* GC-148 (Ezure, 1985)
N-Methyl-1-deoxynojirimycin	*Morus alba* (Moraceae) roots (Asano et al., 1994b)
α-Homonojirimycin (HNJ)	*Omphalea diandra* (Euphorbiaceae) leaves (Kite et al., 1988) *Endospermum medullosum* (Euphorbiaceae) leaves (Kite et al., 1991) *Nephthytis poissoni* (Araceae) leaves (Nash et al., 1996) *Aglaonema treubii* (Araceae) leaves/roots (Asano et al., 1997a) *Hyacinthus orientalis* (Hyacinthaceae) bulb (Asano et al., 1998a)
α-Homonojirimycin-7-*O*-β-D-glucopyranoside (MDL 25,637)	*Omphalea diandra* (Euphorbiaceae) leaves (tentative) (Kite et al., 1990) *Nephthytis poissoni* (Araceae) leaves (Nash et al., 1996) *Aglaonema treubii* (Araceae) leaves/roots (Asano et al., 1997a)

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Table 1 (*continued*)

Alkaloid	Source and reference
	Hyacinthus orientalis (Hyacinthaceae) bulb (Asano et al., 1998a)
	Lobelia sessilifolia (Campanulaceae) whole plant (Ikeda et al., 2000)
α-Homonojirimycin-5-*O*-α-D-galactopyranoside	*Aglaonema treubii* (Araceae) leaves/roots (Asano et al., 1997a)
α-4-*Epi*homonojirimycin	*Aglaonema treubii* (Araceae) leaves/roots (Asano et al., 1997a; Martin et al., 1999)
α-1-Deoxy-1-*C*-methyl-homonojirimycin (Adenophorine)	*Adenophora* spp. (Campanulaceae) roots (Ikeda et al., 2000)
Adenophorine-1-*O*-β-D-glucopyranoside	*Adenophora* spp. (Campanulaceae) roots (Ikeda et al., 2000)
1-Deoxyadenophorine	*Adenophora* spp. (Campanulaceae) roots (Ikeda et al., 2000)
5-Deoxyadenophorine	*Adenophora* spp. (Campanulaceae) roots (Ikeda et al., 2000)
5-Deoxyadenophorine-1-*O*-β-D-glucopyranoside	*Adenophora* spp. (Campanulaceae) roots (Ikeda et al., 2000)
β-1-*C*-butyl-deoxygalactonojirimycin	*Adenophora* spp. (Campanulaceae) roots (Ikeda et al., 2000)
β-Homonojirimycin	*Aglaonema treubii* (Araceae) leaves/roots (Asano et al., 1997a)
	Hyacinthus orientalis (Hyacinthaceae) bulb (Asano et al., 1998a)
β-4,5-Di*epi*homonojirimycin	*Aglaonema treubii* (Araceae) leaves/roots (Asano et al., 1998b)
Nojirimycin B (Mannojirimycin)	*Streptomyces lavandulae* SF-425 (Niwa et al., 1984)
1-Deoxymannojirimycin (DMJ)	*Lonchocarpus sericeus* (Leguminosae) seeds/leaves (Fellows et al., 1979)
	Streptomyces lavandulae GC-148 (Ezure et al., 1988]
	Omphalea diandra (Euphorbiaceae) leaves (Kite et al., 1988)
	Endospermum medullosum (Euphorbiaceae) leaves (Kite et al., 1991)
	Derris malaccensis (Leguminosae) (Asano et al., 1994c)
	Angylocalyx spp. (Leguminosae) seeds/leaves/bark (Nash et al., 1996)
	Hyacinthus orientalis (Hyacinthaceae) bulb (Asano et al., 1998a)
	Adenophora triphylla var. *japonica* (Campanulaceae) whole plant (Asano et al., 2000b)
α-Homomannojirimycin	*Aglaonema treubii* (Araceae) leaves/roots (Asano et al., 1997a)
	Hyacinthus orientalis (Hyacinthaceae) bulb (Asano et al., 1998a)
β-Homomannojirimycin	*Aglaonema treubii* (Araceae) leaves/roots (Asano et al., 1997a)
	Hyacinthus orientalis (Hyacinthaceae) bulb (Asano et al., 1998a)
Galactostatin	*Streptomyces lydicus* PA-5726 (Miyake and Ebata, 1988)
Fagomine	*Fagopyrum esculentum* (Polygonaceae) seeds (Koyama and Sakamura, 1974)
	Xanthocercis zambesiaca (Leguminosae) seeds (Evans et al., 1985b), leaves/roots [Kato et al., 1997a)
	Morus bombycis (Moraceae) leaves (Asano et al., 1994a)
	Morus alba (Moraceae) roots (Asano et al., 1994b)
	Lycium chinense (Solanaceae) roots (Asano et al., 1997b)
3-*Epi*fagomine	*Morus alba* (Moraceae) roots (Asano et al., 1994b)
	Xanthocercis zambesiaca (Leguminosae) leaves/ roots (Kato et al., 1997a)
3,4-Di*epi*fagomine	*Xanthocercis zambesiaca* (Leguminosae) leaves/roots (Kato et al., 1997a)
Fagomine-4-*O*-β-D-glucopyranoside	*Xanthocercis zambesiaca* (Leguminosae) seeds (Evans et al., 1985b) leaves/roots (Kato et al., 1997a)
Fagomine-3-*O*-β-D-glucopyranoside	*Xanthocercis zambesiaca* (Leguminosae) leaves/roots (Kato et al., 1997a)

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Table 1 (*continued*)

Alkaloid	Source and reference
6-Deoxy-fagomine	*Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)
α-1-*C*-Ethyl-fagomine	*Adenophora triphylla* var. *japonica* (Campanulaceae) whole plant (Asano et al., 2000b)
Pyrrolizidines (Fig. 5)	
Alexine	*Alexa* spp. (Leguminosae) seeds/leaves (Nash et al., 1988a)
3,7a-Di*epi*alexine	*Castanospermum australe* (Leguminosae) seeds/leaves (Nash et al., 1988b)
7a-*Epi*alexine (Australine)	*Castanospermum australe* (Leguminosae) seeds/leaves (Molyneux et al., 1988)
1,7a-Di*epi*alexine	*Alexa* spp. and *Castanospermum australe* (Leguminosae) seeds/leaves (Nash et al., 1990a)
7a-*Epi*alexaflorine	*Alexa grandiflora* (Leguminosae) leaves (de S. Pereira et al., 1991)
Casuarine	*Casuarina equisetifolia* (Casuarinaceae) bark (Nash et al., 1994) *Eugenia jambolana* (Myrtaceae) leaves (Wormald et al., 1996)
Casuarine-6-*O*-α-D-glucopyranoside	*Casuarina equisetifolia* (Casuarinaceae) bark (Nash et al., 1994) *Eugenia jambolana* (Myrtaceae) leaves (Wormald et al., 1996)
(1*S*, 2*R*, 3*R*, 7a*R*)-1,2-Dihydroxy-3-hydroxymethylpyrrolizidine (Hyacinthacine A_1)	*Muscari armeniacum* (Hyacinthaceae) bulbs (Asano et al., 2000a)]
(1*R*, 2*R*, 3*R*, 7a*R*)-1,2-Dihydroxy-3-hydroxymethylpyrrolizidine (Hyacinthacine A_2)	*Muscari armeniacum* (Hyacinthaceae) bulbs (Asano et al., 2000a)
(1*R*, 2*R*, 3*R*, 5*R*, 7a*R*)- 1,2-Dihydroxy-3-hydroxymethyl-5-methylpyrrolizidine (Hyacinthacine A_3)	*Muscari armeniacum* (Hyacinthaceae) bulbs (Asano et al., 2000a)
(1*S*, 2*R*, 3*R*, 5*R*, 7a*R*)-1,2-Dihydroxy-3,5-dihydroxy-methylpyrrolizidine (Hyacinthacine B_1)	*Hyacinthoides non-scripta* (Hyacinthaceae) fruits/stalks (Kato et al., 1999) *Scilla campanulata* (Hyacinthaceae) bulb (Kato et al., 1999)
(1*S*, 2*R*, 3*R*, 5*S*, 7a*R*)-1,2-Dihydroxy-3,5-dihydroxy-methylpyrrolizidine (Hyacinthacine B_2)	*Scilla campanulata* (Hyacinthaceae) bulb (Kato et al., 1999)
(1*S*, 2*R*, 3*R*, 5*R*, 7*R*, 7a*R*)-3-Hydroxymethyl-5-methyl-1,2,7-trihydroxypyrrolizidine (Hyacinthacine B_3)	*Muscari armeniacum* (Hyacinthaceae) bulbs (Asano et al., 2000a)
(1*S*, 2*R*, 3*R*, 5*R*, 6*R*, 7*R*, 7a*R*)-3-Hydroxymethyl-5-methyl-1,2,6,7-tetrahydroxypyrrolizidine (Hyacinthacine C_1)	*Hyacinthoides non-scripta* (Hyacinthaceae) fruits/stalks (Kato et al., 1999) *Muscari armeniacum* (Hyacinthaceae) bulbs (Asano et al., 2000a)
(1*R*, 2*R*, 3*R*, 5S, 8*R*)-1,2-Dihydroxy-3-hydroxymethyl-5-[(1*R*)-1,10-dihydroxy-6-oxo-decyl]-pyrrolizidine (Broussonetine N)	*Broussonetia kazinoki* (Moraceae) branches (Shibano et al., 1999b)
Indolizidines (Fig. 6)	
Swainsonine	*Swainsona canescens* (Leguminosae) leaves (Colegate et al., 1979) *Astragalus* spp. (Leguminosae) leaves/stems (Molyneux and James, 1982) *Oxytropis* spp. (Leguminosae) leaves/stems (Molyneux and James, 1982) *Rhizoctonia leguminicola* (Basidiomycetes) (Schneider et al., 1983) *Metarhizium anisopliae* (Deuteromycetes) (Hino et al., 1985) *Ipomoea* sp. aff. *calobra* (Convolvulaceae) seeds (Molyneux et al., 1995) *Ipomoea carnea* (Convolvulaceae) leaves/stems (de Balogh et al., 1999)
Swainsonine *N*-oxide	*Astragalus lentiginosus* (Leguminosae) (Molyneux and James, 1982)

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Table 1 (*continued*)

Alkaloid	Source and reference
Lentiginosine	*Astragalus lentiginosus* (Leguminosae) leaves (Pastuszak et al., 1990)
2-*Epi*lentiginosine	*Rhizoctonia leguminicola* (Basidiomycetes) (Harris et al., 1987) *Astragalus lentiginosus* (Leguminosae) leaves (Pastuszak et al., 1990)
Castanospermine	*Castanospermum australe* (Leguminosae) seeds/leaves/bark (Hohenschutz et al., 1981) *Alexa* spp. (Leguminosae) seeds/leaves/bark (Nash et al., 1988c)
6-*Epi*castanospermine	*Castanospermum australe* (Leguminosae) seeds/leaves/bark (Molyneux et al., 1986; Nash et al., 1990b)
6,7-Di*epi*castanospermine	*Castanospermum australe* (Leguminosae) seeds (Molyneux et al., 1991)
7-Deoxy-6-*epi*castanospermine	*Castanospermum australe* (Leguminosae) seeds (Molyneux et al., 1990)
Nortropanes (Fig. 7)	
Calystegine A_3 and Calystegine B_2	*Calystegia sepium* (Convolvulaceae) leaves/roots (Tepfer et al., 1988; Goldmann et al., 1990) *Convolvulus arvensis* (Convolvulaceae) leaves/roots (Tepfer et al., 1988; Molyneux et al., 1993b) *Atropa belladonna* (Solanaceae) leaves/roots (Tepfer et al., 1988; Goldmann et al., 1990; Dräger et al., 1995) *Solanum* spp. (Solanaceae) tubers/leaves (Nash et al., 1993) *Ipomoea batatus* (Convolvulaceae) leaves/roots (Nash et al., 1996) *Datura wrightii* (Solanaceae) leaves (Nash et al., 1993) *Physalis alkekengi* var. *francheti* (Solanaceae) roots (Asano et al., 1995a) *Hyoscyamus niger* (Solanaceae) leaves/roots (Dräger et al., 1995) *Mandragora officinarum* (Solanaceae) leaves/roots/fruits (Dräger et al., 1995) *Scopolia* spp. (Solanaceae) leaves/roots (Dräger et al., 1995; Asano et al., 1996a) *Ipomoea* sp. *aff. calobra* (Convolvulaceae) seeds (Molyneux et al., 1995) *Calystegia japonica* (Convolvulaceae) roots (Nash et al., 1996) *Lycium chinense* (Solanaceae) roots (Asano et al., 1997b) B_2 only in *Morus alba* (Moraceae) fruits (Asano et al., 1997c) *Ipomoea carnea* (Convolvulaceae) leaves/stems (de Balogh et al., 1999)
N-Methyl-calystegine B_2	*Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)
Calystegine A_5	*Physalis alkekengi* var. *francheti* (Solanaceae) (Asano et al., 1995a) *Scopolia japonica* (Solanaceae) roots (Asano et al., 1996a) *Hyoscyamus niger* (Solanaceae) leaves/roots (Asano et al., 1996b) *Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)
Calystegine A_6	*Hyoscyamus niger* (Solanaceae) leaves/roots (Asano et al., 1996b) *Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)
Calystegine A_7	*Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)
Calystegine B_1	*Convolvulus arvensis* (Convolvulaceae) leaves/roots (Tepfer et al., 1988; Molyneux et al., 1993b) *Calystegia sepium* (Convolvulaceae) leaves/roots (Goldmann et al., 1990)

(*continued on next page*)

Table 1 (*continued*)

Alkaloid	Source and reference
	Physalis alkekengi (Solanaceae) roots (Asano et al., 1995a) *Hyoscyamus niger* (Solanaceae) leaves/roots (Dräger et al., 1995) *Mandragora officinarum* (Solanaceae) leaves/roots/fruits (Dräger et al., 1995) *Scopolia* spp. (Solanaceae) leaves/roots (Dräger et al., 1995; Asano et al., 1996a) *Ipomoea batatus* (Convolvulaceae) leaves/roots (Nash et al., 1996; Asano et al., 1997c) *Duboisia leichhardtii* (Solanaceae) leaves (Kato et al., 1997b) *Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)
Calystegine B_1-3-*O*-β-D-glucopyranoside	*Nicandra physalodes* (Solanaceae) fruits (Griffiths et al., 1996)
Calystegine B_3	*Physalis alkekengi* (Solanaceae) roots (Asano et al., 1995a) *Scopolia japonica* (Solanaceae) roots (Asano et al., 1996a) *Hyoscyamus niger* (Solanaceae) leaves/roots (Asano et al., 1996b) *Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)
Calystegine B_4	*Scopolia japonica* (Solanaceae) roots (Asano et al., 1996a) *Duboisia leichhardtii* (Solanaceae) leaves (Kato et al., 1997b) *Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)
Calystegine B_5	*Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)
Calystegine C_1	*Morus alba* (Moraceae) roots (Asano et al., 1994b) *Ipomoea batatus* (Convolvulaceae) roots (Nash et al., 1996) *Scopolia japonica* (Solanaceae) roots (Asano et al., 1996a) *Duboisia leichhardtii* (Solanaceae) leaves (Kato et al., 1997b) *Lycium chinense* (Solanaceae) roots (Asano et al., 1997b) *Ipomoea carnea* (Convolvulaceae) leaves/stems (de Balogh et al., 1999)
N-Methyl-calystegine C_1	*Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)
Calystegine C_2	*Duboisia leichhardtii* (Solanaceae) leaves (Kato et al., 1997b) *Lycium chinense* (Solanaceae) roots (Asano et al., 1997b]
Calystegine N_1	*Hyoscyamus niger* (Solanaceae) leaves/roots (Asano et al., 1996b) *Lycium chinense* (Solanaceae) roots (Asano et al., 1997b)

producing polyhydroxylated alkaloids which could then be mistakenly considered of plant origin.

From Table 1, it can be seen that the majority of naturally-occurring polyhydroxylated alkaloids have been isolated from plants. Although the first alkaloid of this type to be discovered (nojirimycin) was isolated from the fermentation broth of a species of *Streptomyces*, only a limited number of bacteria (principally Actinomycetes) have subsequently been found that produce polyhydroxylated alkaloids. Also, only three of the compounds listed in Table 1 are known to be produced by fungi (nectrisine, swainsonine and 2-*epi*lentiginosine). However, the apparent restricted distribution of polyhydroxylated alkaloids amongst micro-organisms may be misleading as the techniques used to screen the cultures are frequently inappropriate for detecting such highly polar compounds. However, there are actually quite a range of microbial products that can be described as carbohydrate analogues and some of these with therapeutic potential are discussed in Section 5.1.

The recent increase in the rate at which novel water-soluble alkaloids are reported would suggest that many more await discovery from diverse sources, including plants and micro-organisms in many taxa not previously considered to be alkaloid producers. Several hundred related alkaloids have also been synthesised which have allowed investigations of structure-activity relationships, but it is now becoming apparent that many of these synthetic structures also occur naturally. For example, the 7-*O*-β-D-glucopyranosyl derivative of α-homonojirimycin was designed to be a transition state analogue of sucrose (Liu, 1987) before it and its aglycone were discovered as

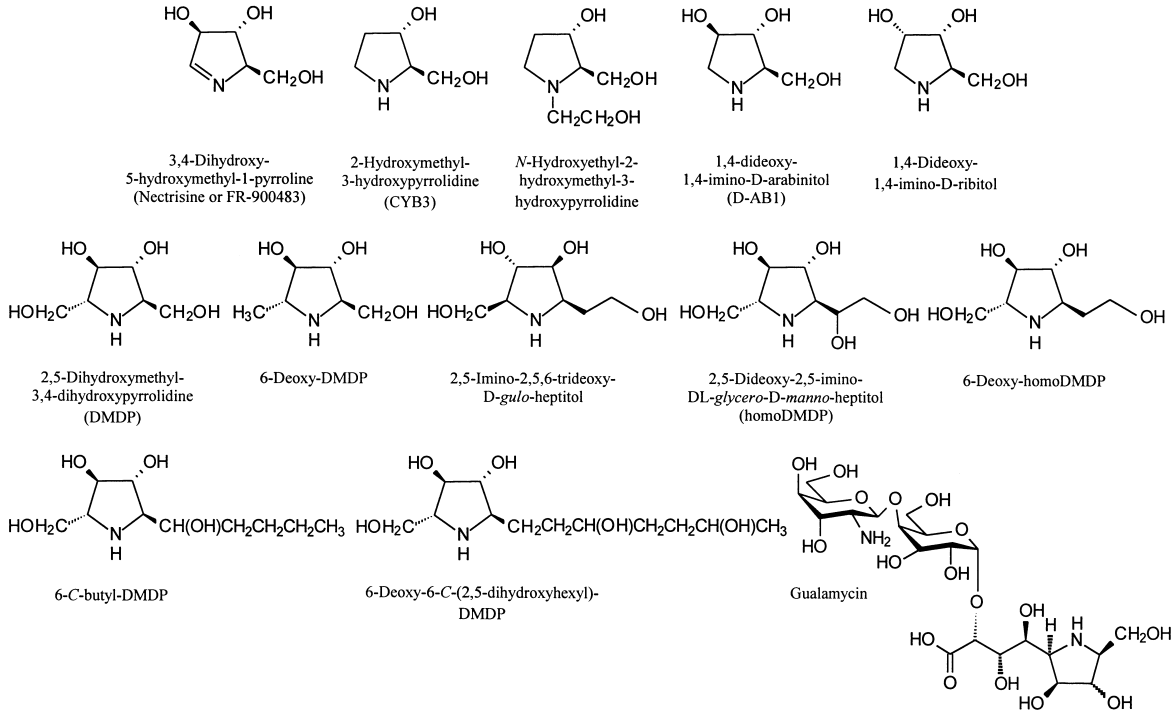

Fig. 2. Some naturally-occurring polyhydroxylated pyrroline and pyrrolidine alkaloids.

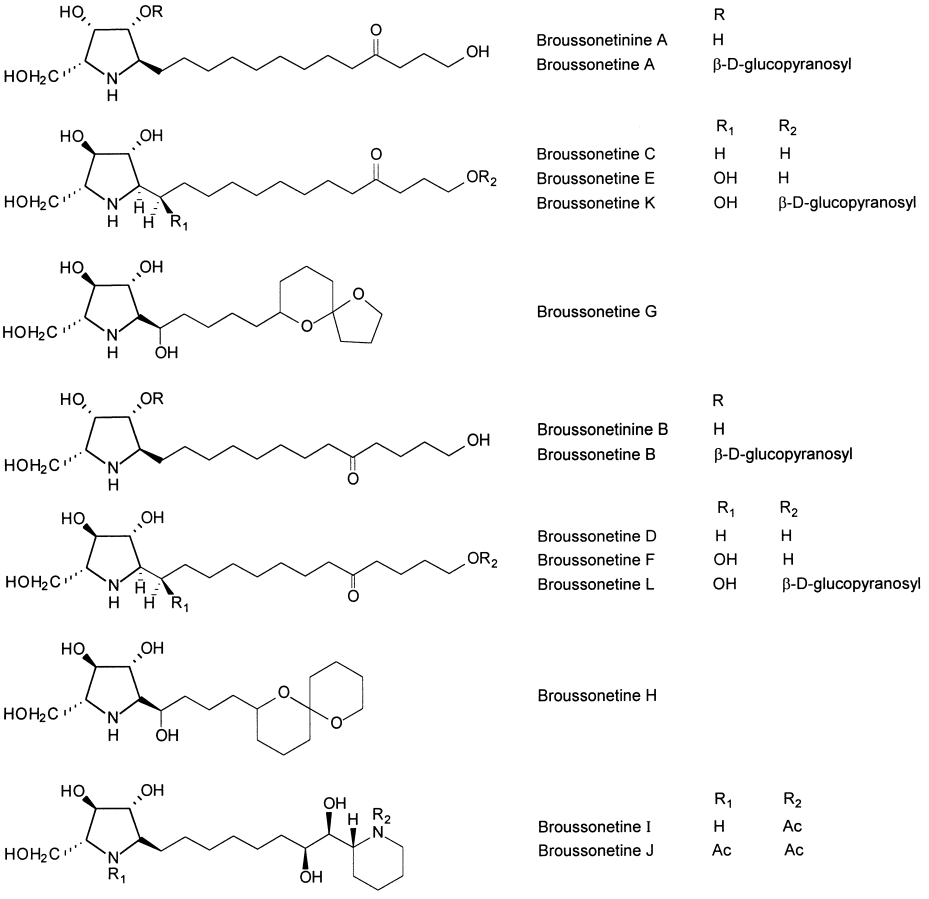

Fig. 3. Polyhydroxylated pyrrolidine alkaloids and derivatives isolated from *Broussonetia kazinoki*.

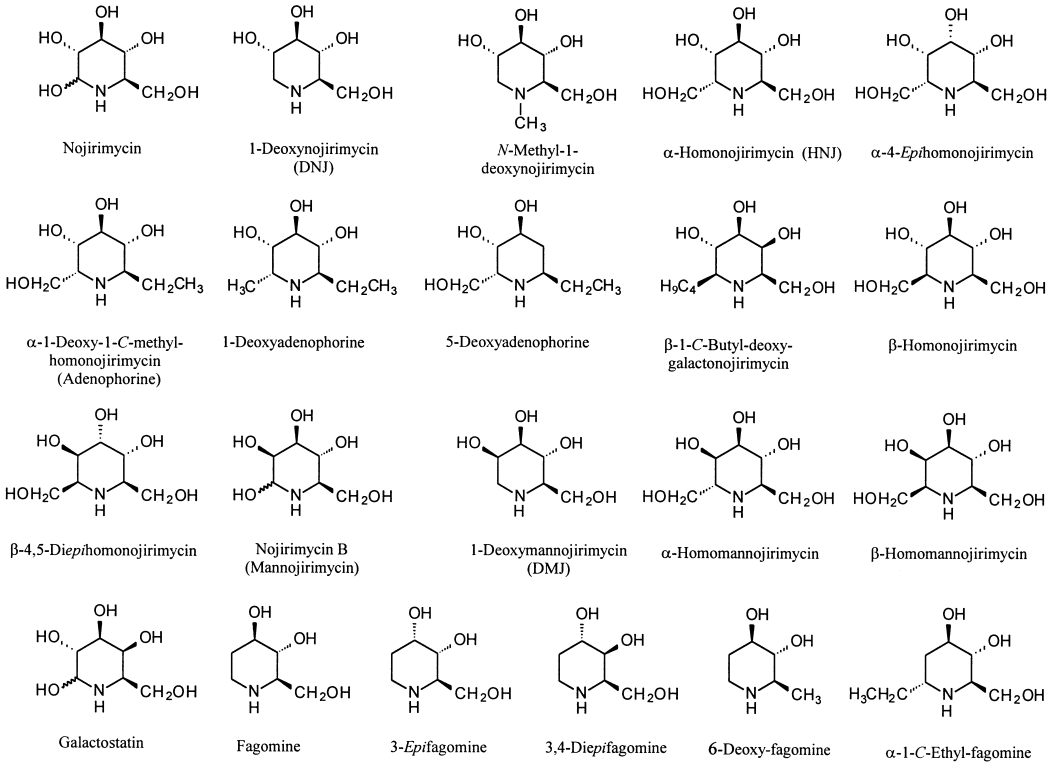

Fig. 4. Some naturally-occurring polyhydroxylated piperidine alkaloids.

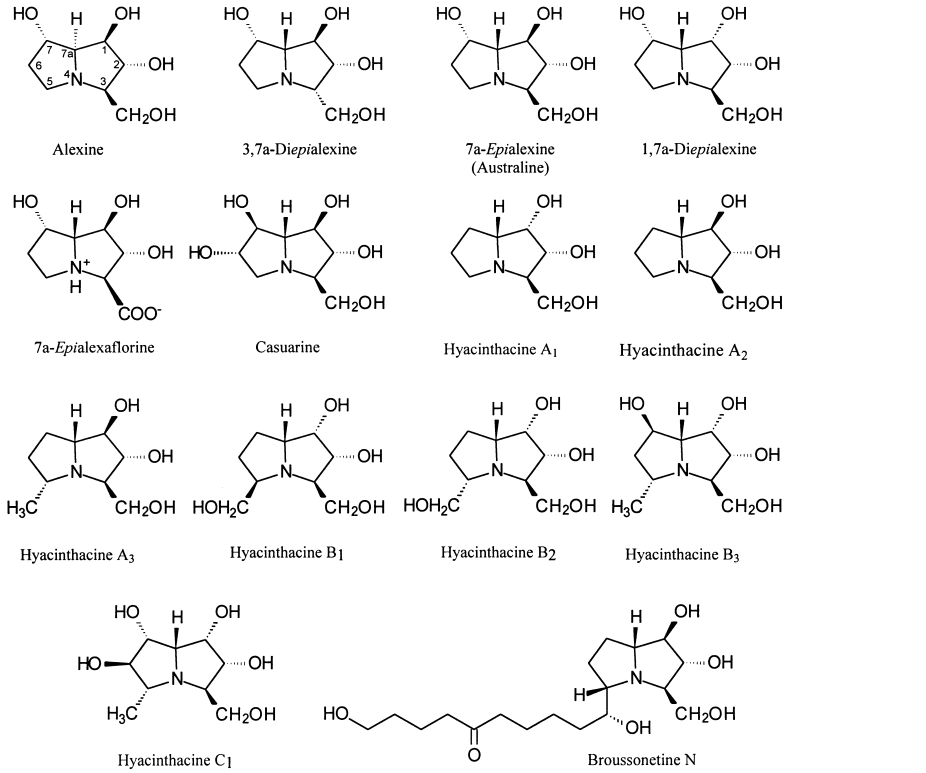

Fig. 5. Some naturally-occurring polyhydroxylated pyrrolizidine alkaloids [recently, Wormald et al. (1998) reported that 7,7a-di*epi*alexine, originally identified as a natural product from *Castanospermum australe* (Nash et al., 1990a) was in fact incorrectly described in the original paper as identification was based on a comparison with erroneous NMR data published for 7a-*epi*alexine by Molyneux et al. (1988). Therefore, 7,7a-di*epi*alexine can no longer be regarded as a natural product and so it is not depicted here].

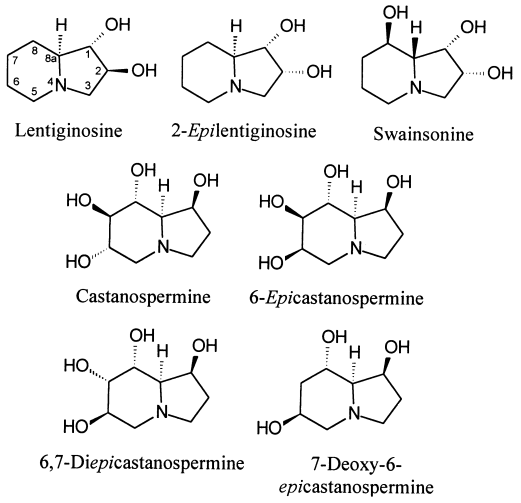

Fig. 6. Some naturally-occurring polyhydroxylated indolizidine alkaloids.

natural products (Kite et al., 1990). Similarly, the synthetic piperidine alkaloids β-homonojirimycin (Holt et al., 1994; Martin and Saavedra, 1995), α-homomannojirimycin (Bruce et al., 1992) and β-homomannojirimycin (Holt et al., 1994) were all recently isolated from *Aglaonema treubii* (Araceae) (Asano et al., 1997a).

3. Polyhydroxylated alkaloids as glycosidase inhibitors

Most of the polyhydroxylated alkaloids listed in Section 2 that have been studied in detail have been shown to inhibit glycosidases in a reversible and competitive manner (Legler, 1990; Asano et al., 1995b). Glycosidases are enzymes that catalyse the hydrolysis of the glycosidic bonds in complex carbohydrates and glycoconjugates. The wide variety of functions in which glycosidases are involved makes them essential for the survival and existence of all living organisms. For example, digestive glycosidases break down large sugar-containing molecules to release monosaccharides which can be more easily taken up and used metabolically by the organism; lysosomal glycosidases catabolise glycoconjugates intracellularly; and a wide range of glycosidases are involved in the biosynthesis of the oligosaccharide portions of glycoproteins and glycolipids which play vital roles in mammalian cellular structure and function. Thus, membrane glycoproteins include receptors for biologically important molecules such as hormones, low-density lipoprotein or acetylcholine whilst others are involved in cell–cell adhesion. The oligosaccharide chains play an important role in the correct functioning of these proteins by stabilising them and ensuring that they have the correct conformation and they may also be involved in the targeting mechanism of certain proteins (Elbein, 1989).

Since the mode of action of glycosidases involves the cleavage of glycosidic bonds between sugar molecules, individual glycosidases show specificity for certain sugar molecules and for a specific anomeric configuration of that sugar. Polyhydroxylated alkaloids can be extremely potent and specific inhibitors of glycosidases by mimicking the pyranosyl or furanosyl moiety of their natural substrates. Therefore, the number, position and configuration of the hydroxyl groups of each alkaloid dictate the type of glycosidases which are inhibited. For example, the configuration of the hydroxyl substituents of the glucosidase inhibitor nojirimycin correspond to those of glucose in the pyranose configuration. Inouye et al. (1968) reported that nojirimycin exists in aqueous

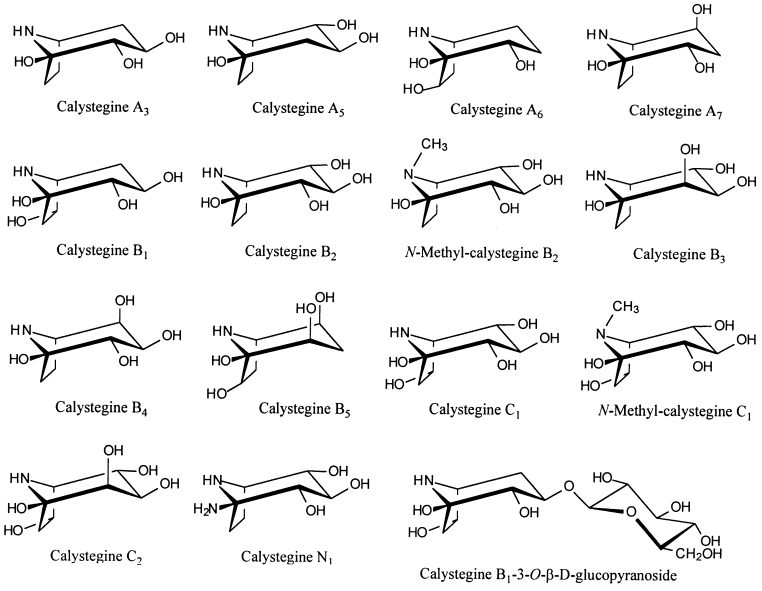

Fig. 7. Some naturally-occurring polyhydroxylated *nor*tropane alkaloids.

solution in both α- and β- forms with an equilibrium of 60% of the former and 40% of the latter and each of these forms are responsible for the inhibition of α- and β-glucosidases, respectively. Although the spatial arrangement of the hydroxyl groups of polyhydroxylated alkaloids serves as a means of recognition by specific glycosidases, it is the influence of the endocyclic nitrogen atom on the conformation and electrostatic properties of the molecule that is important for inhibition of enzyme activity. It has been suggested that when a polyhydroxylated alkaloid binds to the active site of a glycosidase, protonation of the compound leads to the formation of an ion pair between the inhibitor and a carboxylate anion in the active site of the enzyme (Legler, 1990). The protonated inhibitor closely resembles the transition state of the natural substrate and hence the enzyme has a high affinity for the molecule. However, the strength of the binding and hence the effectiveness of the inhibition depends to a degree on the p*K*a of the inhibitor and the pH optimum of the enzyme (Lalegerie et al., 1982).

Although the nature of the glycosidases that will be inhibited by certain polyhydroxylated alkaloids can be predicted, to some extent, from the number, position and configuration of the hydroxyl groups, there can be marked differences in the inhibition of isoenzymes of a given glycosidase in different species (Scofield et al., 1995a,b) and even within the same cell. For example, in human liver cells there are multiple forms of α-mannosidase which are structurally, functionally and genetically quite distinct (Winchester, 1984). They also have different subcellular locations. The lysosomal α-mannosidase, which catabolises glycoconjugates, has an acidic pH optimum (around 4.0) whereas the cytosolic α-mannosidase functions best at near neutral pH (6.5). There are also two forms of α-mannosidase in the Golgi (α-mannosidase I and II, respectively) which are involved in glycoprotein processing, as is another α-mannosidase which is associated with the endoplasmic reticulum. The lysosomal, Golgi α-mannosidase II and neutral α-mannosidases are all inhibited by polyhydroxylated alkaloids with the same substituents and chirality as mannofuranose (e.g. swainsonine) whereas Golgi α-mannosidase I is inhibited by mannopyranose analogues such as DMJ (Winchester et al., 1993).

There is now a vast amount of literature on the inhibition of glycosidases by particular polyhydroxylated alkaloids and related compounds (both natural and synthetic) and an overview of this area is given in some reviews (Legler, 1990; Winchester and Fleet, 1992; Asano et al., 1995b). It should be noted, however, that the polyhydroxylated alkaloids discussed above are all inhibitors of exoglycosidases, which cleave the glycosidic linkage at the non-reducing terminus of the saccharide chain, liberating monosaccharide units. However, certain animal cells also possess endoglycosidases which act on glycosidic linkages within saccharide chains giving rise to smaller oligosaccharide units. Endoglycosidases are not generally inhibited by polyhydroxylated alkaloids. A large amount of research has been conducted on the inhibition of exoglycosidases involved in glycoprotein formation and catalysis which has been shown to be important in both the toxicity and the potential therapeutic uses of the polyhydroxylated alkaloids (Sections 4 and 5). Table 2 lists some of the exoglycosidases that the polyhydroxylated alkaloids are reported to inhibit. This is not a comprehensive list, as not all naturally-occurring polyhydroxylated alkaloids have been tested on a wide range of glycosidases. It should also be remembered that due to the variations in specificity of isoenzymes, both between species and within the same cell, it is difficult to predict, for example, that a potent inhibitor of certain α-D-glucosidases will inhibit all α-D-glucosidases as strongly, if at all.

4. Mammalian toxicity of polyhydroxylated alkaloids

As might be expected for a group of compounds that affect carbohydrate metabolism, the polyhydroxylated alkaloids have been reported to have a wide range of effects on a number of organisms and some cause serious livestock poisonings. In fact, it was the toxicity to livestock of the legumes *Swainsona canescens* and *Castanospermum australe* in Australia that first led to the isolation of the toxic principles swainsonine (Colegate et al., 1979) and castanospermine (Hohenschutz et al., 1981).

Cattle eating *Swainsona* species developed a syndrome called "pea struck" which is due to decreased α-mannosidase activity resulting in accumulation of mannose-rich oligosaccharides in lysosomes. This results in neuronal vacuolation, axonal dystrophy, loss of cellular function and ultimately death (Dorling et al., 1978). The disorder "locoism" in the western United States was also found to be due to swainsonine in locoweeds (*Astragalus* and *Oxytropis* species), (Molyneux and James, 1982; Molyneux et al., 1985). The clinical symptoms of swainsonine poisoning in livestock on ingestion of plants containing this compound include depression, tremors, nervousness, emaciation, gastrointestinal malfunction and reproductive alterations such as abortion and birth defects (Stegelmeier et al., 1995a). Poisoning of animals in China by *Oxytropis ochrocephala* and *Oxytropis kansuensis* has also been shown to be due to the presence of swainsonine (Cao et al., 1992).

The concentration of swainsonine detected in all plants implicated in poisonings is extremely low. For example, Molyneux and James (1982) isolated swainsonine from the vegetative parts of *Astragalus lentiginosus* in a yield of 0.007% (dry weight) and the levels detected in *O. ochrocephala* and *O. kansuensis* were 0.012 and 0.021% (dry weights), respectively (Cao et al., 1992).

Table 2
A list of some of the exoglycosidases that have been found to be inhibited by naturally-occurring polyhydroxylated alkaloids

Alkaloid	Glycosidases inhibited
Pyrroline	
Nectrisine (FR-900483)	α-Glucosidase, α-mannosidase (Shibata et al., 1988; Kayakiri et al., 1991)
Pyrrolidines	
2*R*-Hydroxymethyl-3*S*-hydroxypyrrolidine (CYB-3)	Weak inhibitor of isomaltase (Scofield et al., 1986)
1,4-Dideoxy-1,4-imino-D-arabinitol (D-AB1)	Potent inhibitor of α-glucosidases (including maltase, isomaltase, sucrase and trehalase, Glucosidase II), α-D-arabinosidase, β-glucosidases (including lactase and cellobiase) and α-mannosidases (Mannosidase II and lysosomal). (Fleet et al., 1985; Scofield et al., 1986; Axamawaty et al., 1990; Asano et al., 1995b)
1,4-Dideoxy-1,4-imino-(2-*O*-β-D-glucopyranosyl)-D-arabinitol	Weak inhibitor of α- and β-glucosidases (Asano et al., 1994b)
1,4-Dideoxy-1,4-imino-D-ribitol	Weak inhibitor of α-glucosidase (Winchester and Fleet, 1992)
2*R*,5*R*-Dihydroxymethyl-3*R*,4*R*-dihydroxy-pyrrolidine (DMDP)	β-Glucosidases (including cellobiase) and α-glucosidases (including invertase, sucrase, isomaltase, trehalase), human lysosomal β-mannosidase, β-galactosidase (including lactase) and β-xylosidase (Evans et al., 1985a; Fellows et al., 1986; Scofield et al., 1995a and b; Asano et al., 1995b)
6-Deoxy-DMDP	β-Mannosidase, β-galactosidase and α-fucosidase (Molyneux et al., 1993a)
6-*C*-Butyl-DMDP	Good β-glucosidase inhibitor and poor inhibitor of β-galactosidase (Asano et al., 2000b)
6-Deoxy-6-*C*-(2,5-dihydroxyhexyl)-DMDP	Moderate inhibitor of amyloglucosidase and weak inhibitor of α-fucosidase only (Kato et al., 1999)
2,5-Imino-2,5,6-trideoxy-D-*gulo*-heptitol	Moderate inhibitor of α-fucosidase (Asano et al., 1998a)
2,5-Dideoxy-2,5-imino-DL-*glycero*-D-*manno*-heptitol (homo-DMDP)	Potent inhibitor of β-glucosidases and β-galactosidases (including lactase) and trehalase, moderate inhibitor of other α-glucosidases (including maltase) and α- and β-mannosidases (Watson et al., 1997; Kato et al., 1999)
Homo-DMDP-7-*O*-apioside	Good inhibitor of β-galactosidases (including lactase) and amyloglucosidase (Kato et al., 1999)
Homo-DMDP-7-*O*-β-D-xylopyranoside	Potent inhibitor of β-glucosidases and β-galactosidases (including lactase) and moderate inhibitor of amyloglucosidase and β-mannosidase (Kato et al., 1999)
6-Deoxy-homoDMDP	Potent inhibitor of α-glucosidases (including maltase) (Asano et al., 1998a)
Broussonetines A and B	Not inhibitory (Shibano et al., 1997b)
Broussonetinines A and B	Potent inhibitors of β-galactosidase and α-mannosidase (Shibano et al., 1997b)
Broussonetines C and D	Potent inhibitors of β-galactosidase and β-mannosidase (Shibano et al., 1997a)
Broussonetines E and F	Potent inhibitors of β-glucosidase, β-galactosidase and β-mannosidase and good inhibitors of α-glucosidase (Shibano et al., 1997b)
Broussonetines G, H, K and L	Potent inhibitors of β-glucosidase, β-galactosidase and β-mannosidase (Shibano et al., 1998a, 1999a)
Piperidines	
Nojirimycin	β-Glucosidases and α-glucosidases (including sucrase, maltase, isomaltase and amylase), β-galactosidases and *N*-acetyl-β-D-glucosaminidases (Fuhrmann et al., 1985; Legler, 1990)
1-Deoxynojirimycin (DNJ)	Potent inhibitor of α-glucosidases (including trehalase, invertase, sucrase, maltase, isomaltase), more weakly inhibitory to Glucosidase I and II, and β-glucosidases, also α-mannosidases, α-fucosidase, α- and β-galactosidases (including lactase) (Evans et al., 1985a; Fellows et al., 1986; Legler, 1990; Asano et al., 1995b)
1-Deoxynojirimycin-2-*O*-, 3-*O*-, 4-*O*-α-D-glucopyranosides and 2-*O*-, 6-*O*-α-D-galactopyranoside and 2-*O*-, 3-*O*-, 4-*O*-, 6-*O*-β-D-glucopyranoside	Varying levels of inhibition of α-glucosidases (Asano et al., 1994b)
N-Methyl-1-deoxynojirimycin	More potent inhibitor of α-glucosidases than DNJ (Asano et al., 1995b)

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Table 2 (*continued*)

Alkaloid	Glycosidases inhibited
α-Homonojirimycin (HNJ)	α-Glucosidases (including maltase, sucrase and trehalase), β-glucosidases, lactase and α-galactosidases (Kite et al., 1988, 1990) and moderate inhibitor of Glucosidase I and II (Zeng et al., 1997)
α-Homonojirimycin-7-*O*-β-D-glucopyranoside (MDL 25,637)	α-Glucosidases (including Glucosidase II and trehalase) (Kyosseva et al., 1995)
α-4-*Epi*homonojirimycin	Poor inhibitor of α- and β-galactosidases (Asano et al., 1998b; Martin et al., 1999)
α-1-Deoxy-1-*C*-methyl-homonojirimycin (Adenophorine)	Good α-galactosidase inhibitor and moderate to poor inhibitor of α-glucosidases (Ikeda et al., 2000)
Adenophorine-1-*O*-β-D-glucopyranoside	Good trehalase inhibitor but moderate to poor inhibitor of other α-glucosidases (Ikeda et al., 2000)
1-Deoxyadenophorine	Good α-galactosidase inhibitor and moderate to poor inhibitor of α-glucosidases (Ikeda et al., 2000)
5-Deoxyadenophorine	Good α- and β-galactosidase inhibitor and poor trehalase inhibitor (Ikeda et al., 2000)
5-Deoxyadenophorine-1-*O*-β-D-glucopyranoside	Potent inhibitor of α-glucosidases and α-galactosidases (Ikeda et al., 2000)
β-1-*C*-Butyl-deoxygalactonojirimycin	Potent α-galactosidase inhibitor only (Ikeda et al., 2000)
β-Homonojirimycin	Weak inhibitor of β-glucosidase (Asano et al., 1997a)
β-4,5-Di*epi*homonojirimycin	Potent inhibitor of α-glucosidases (including maltase and sucrase) and α-galactosidase, moderate inhibitor of α-fucosidase (Asano et al., 1998b)
Nojirimycin B (Mannojirimycin)	α-mannosidases (Niwa et al., 1984)
1-Deoxymannojirimycin (DMJ)	α-Mannosidases (Mannosidase I) and α-fucosidase (Ishida et al., 1967; Bischoff and Kornfeld, 1984; Evans et al., 1985a)
α-Homomannojirimycin	Weak inhibitor of human liver α-mannosidases (Asano et al., 1997a)
β-Homomannojirimycin	β-Mannosidase (Asano et al., 1997a)
Galactostatin	β-Galactosidases (Miyake and Ebata, 1988)
Fagomine	Weak inhibitor of α-glucosidases (including isomaltase and sucrase) (Kato et al., 1997a)
3-E*pi*fagomine	Isomaltase, β-galactosidases and lactase (Kato et al., 1997a)
3,4-Di*epi*fagomine	Not inhibitory (Kato et al., 1997a)
Fagomine-4-*O*-β-D-glucopyranoside	Weak inhibitor of β-galactosidase (Scofield et al., 1986; Kato et al., 1997a)
Fagomine-3-*O*-β-D-glucopyranoside	Not inhibitory (Kato et al., 1997a)
α-1-*C*-Ethyl-fagomine	Good β-galactosidase inhibitor and poor inhibitor of α-glucosidases (Asano et al., 2000b)
Pyrrolizidines	
Alexine	Disaccharidase-type α-glucosidases (trehalase, amyloglucosidase) and β-galactosidase (Nash et al., 1988a, 1990a)
3,7a-Di*epi*alexine	Disaccharidase-type α-glucosidases (amyloglucosidase and sucrase) (Nash et al., 1990a)
7a-*Epi*alexine (Australine)	Disaccharidase-type α-glucosidases (amyloglucosidase, sucrase, maltase) and Glucosidase I, β-glucosidase, β-galactosidase (Molyneux et al., 1988; Zeng et al., 1997)
1,7a-Di*epi*alexine	α-glucosidases (amyloglucosidase), weakly inhibitory to β-glucosidases (Nash et al., 1990a)
7a-*Epi*alexaflorine	Weak inhibitor of amyloglucosidase and sucrase (de S. Pereira et al., 1991)
Casuarine	α-Glucosidases (including trehalase, amyloglucosidase and glucosidase I) (Bell et al., 1997)
Hyacinthacine A_1	Potent inhibitor of lactase, moderate inhibitor of amyloglucosidase and α-fucosidase and a poor inhibitor of α- and β-glucosidases and β-mannosidase (Asano et al., 2000a)
Hyacinthacine A_2	Potent inhibitor of amyloglucosidase, good inhibitor of lactase, moderate inhibitor of β-glucosidase and trehalase and weak inhibitor of β-mannosidase (Asano et al., 2000a)
Hyacinthacine A_3	Good inhibitor of amyloglucosidase and moderate inhibitor of lactase (Asano et al., 2000a)

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Table 2 (*continued*)

Alkaloid	Glycosidases inhibited
Hyacinthacine B_1	Moderate to poor inhibitor of β-glucosidases and β-galactosidases (Kato et al., 1999)
Hyacinthacine B_2	Moderate to good inhibitor of β-glucosidases and β-galactosidases (including potent inhibition of lactase) (Kato et al., 1999)
Hyacinthacine B_3	Good inhibitor of lactase and amyloglucosidase and poor inhibitor of α-fucosidase (Asano et al., 2000a)
Hyacinthacine C_1	Moderate inhibitor of amyloglucosidase (Kato et al., 1999)
Broussonetine N	Good inhibitor of β-glucosidase, β-galactosidase and β-mannosidase (Shibano et al., 1999b)
Indolizidines	
Swainsonine	Potent inhibitor of α-mannosidases (Tulsiani et al., 1982; Cenci di Bello et al., 1989a,b)
Lentiginosine	Amyloglucosidase (Pastuszak et al., 1990)
2-*Epi*lentiginosine	Not inhibitory (Pastuszak et al., 1990)
Castanospermine	α-Glucosidases (including amyloglucosidase, sucrase, maltase, isomaltase, trehalase, amylase, Glucosidase I and II) and β-glucosidases (including lactase and cellobiase), β-glucocerebrosidase and β-xylosidase (Saul et al., 1983; Scofield et al., 1995a,b)
6-*Epi*castanospermine	Amyloglucosidase, neutral α-mannosidase (Molyneux et al., 1986; Winchester et al., 1990)
6,7-Di*epi*castanospermine	Amyloglucosidase and fungal β-glucosidase (Molyneux et al., 1991)
7-Deoxy-6-*epi*-castanospermine	Amyloglucosidase and yeast α-glucosidase (Molyneux et al., 1990)
Nortropanes	
Calystegine A_3	Potent inhibitor of β-glucosidases, α- and β-galactosidases (including lactase) and trehalase (Asano et al., 1995a)
Calystegine A_5	Not inhibitory (Asano et al., 1995a)
Calystegine A_6	Moderate inhibitor of trehalase only (Asano et al., 1996b)
Calystegine A_7	Moderate inhibitor of trehalase only (Asano et al., 1997b)
Calystegine B_1	Potent inhibitor of β-glucosidases (including cellobiase) and β-galactosidase (including lactase) and a weak inhibitor of trehalase (Asano et al., 1995a)
Calystegine B_1-3-*O*-β-D-glucopyranoside	Potent inhibitor of rice α-glucosidase and weak inhibitor of β-glucosidases (Asano et al., 1997d)
Calystegine B_2	Potent inhibitor of β-glucosidases, disaccharidase-type α-glucosidases (including maltase, sucrase and trehalase) and α- and β-galactosidase (including lactase) (Asano et al., 1996a)
N-Methyl-calystegine B_2	Potent inhibitor of α-galactosidases and a moderate inhibitor of trehalase (Asano et al., 1997b)
Calystegine B_3	Weak inhibitor of β-glucosidases, α-galactosidases and trehalase (Asano et al., 1995a; Kato et al., 1997b)
Calystegine B_4	Moderate inhibitor of α-galactosidases and trehalase, but weak inhibitor of β-glucosidases (including cellobiase) and lactase (Asano et al., 1996a)
Calystegine C_1	Potent inhibitor of disaccharidase-type α-glucosidases and β-glucosidases, moderate inhibitor of α- and β-galactosidases (Asano et al., 1995a, 1996a; Kato et al., 1997b)
N-Methyl-calystegine C_1	Weak inhibitor of some α-galactosidases (Asano et al., 1997b)
Calystegine C_2	Moderate inhibitor of β-glucosidases, α-mannosidases and trehalase (Kato et al., 1997b)
Calystegine N_1	Weak inhibitor of disaccharidase-type α-glucosidases and potent inhibitor of β-glucosidases (including cellobiase), lactase and α-galactosidase (Asano et al., 1996b)

However, swainsonine can accumulate within the tissues of the body as it has the ability to permeate the plasma membrane freely but once inside lysosomes it becomes protonated due to the low pH and so becomes concentrated (Chotai et al., 1983). Therefore, poisoning by swainsonine generally takes several weeks of ingestion before it becomes apparent. If affected animals are denied access to plants containing swainsonine early enough, lysosomal function will return to normal as the alkaloid is excreted from the body via the urine (Daniel et al., 1984). Although many of the lesions will disappear, some long-term effects may result in reduced animal

performance (Stegelmeier et al., 1995a). A threshold of toxicity is difficult to establish for swainsonine, but Molyneux and co-workers (1994) suggested that a concentration in the diet of as little as 0.001% should be of concern.

Even before swainsonine was identified as the toxic principle in *Swainsona* species, Dorling et al. (1978) recognised that the lysosomal storage disorder induced in animals grazing these plants was biochemically and morphologically similar to the rare genetically-determined mannosidosis that occurs in humans (Ockerman, 1973) and Angus cattle (Hocking et al., 1972). [N.B. Mannosidosis has subsequently also been found to occur naturally in cats (Burditt et al., 1980) and guinea pigs (Crawley et al., 1999).] This disease is characterised by accumulation in cells and excretion in the urine of mannose-rich oligosaccharide which results from a deficiency in all tissues of lysosomal α-D-mannosidase (Jolly et al., 1981). The condition is ultimately fatal. In infants the clinical syndrome may include mental retardation, central nervous system derangement, abnormal musculoskeletal development, locomotor incoordination, abnormal facial appearance and an increased susceptibility to recurrent infections (Ockerman, 1973). However, swainsonine-induced mannosidosis is not an exact model of genetic mannosidosis since this compound inhibits α-mannosidase II in addition to the lysosomal α-mannosidase. Nevertheless, swainsonine has proved a valuable tool in the study of this lysosomal storage disease since it has provided the means of inducing a reversible phenocopy of the genetic disorder in animals and in tissue culture (Cenci di Bello et al., 1983). The use of such models could allow investigation of the viability of enzyme replacement therapy in the treatment of this disease (Winchester, 1992).

Comparison of the sequences of the major oligosaccharides which accumulate and are excreted in the urine of humans suffering from mannosidosis with those excreted in the swainsonine-induced disease has allowed the intracellular substrate specificity of lysosomal α-mannosidase to be defined (Daniel et al., 1992). In 1983, Cenci di Bello and co-workers first realised that the human lysosomal α-mannosidase actually consists of two enzymes which are specific for different mannose linkages. The major enzyme present catalyses α-1,3-linkages but the other is specific for α-1,6-linkages (de Gasperi et al., 1992). In human mannosidosis the α-1,3 activity is absent but the α-1,6 activity is unaffected. Swainsonine inhibits the activity of both enzymes. However, cattle do not possess the α-1,6 form of lysosomal α-mannosidase and so the storage products in the bovine form of genetic mannosidosis bear a greater similarity to those seen in the form of the disease induced by swainsonine (de Gasperi et al., 1992).

Castanospermum australe contains the potent α- and β-glucosidase inhibitor castanospermine at a concentration of approximately 0.3% of the dry weight of the seed (Hohenschutz et al., 1981). Pigs, cattle and horses have been reported to be poisoned when they have consumed the seeds of *C. australe*. The chief symptom is gastroenteritis, owing to inhibition of the activity of intestinal brush border digestive disaccharidases (e.g. sucrase, maltase and trehalase) (McKenzie et al., 1988). The upset can be so severe that the animals can die (Pan et al., 1993). Other symptoms include myocardial degeneration and nephrosis (McKenzie et al., 1988). Several cases of human poisoning have also occurred (Everist, 1974). Histologic changes observed in poisoned livestock and rodent feeding trials include degenerative vacuolation of hepatocytes and skeletal myocytes (McKenzie et al., 1988; Stegelmeier et al., 1995a,b). Castanospermine inhibits the activity of lysosomal α-glucosidase which leads to the accumulation of glycogen within the lysosomes (Saul et al., 1985). This mimics the situation in the genetically-determined storage disorder glycogenosis type II (Pompe's disease) which is caused by a deficiency of lysosomal α-glucosidase (Glew et al., 1985). However, castanospermine also inhibits the activity of lysosomal β-glucosidase and this affects the catabolism of glycosphingolipids (Cenci di Bello et al., 1988).

Recently, swainsonine has been detected in species of the Convolvulaceae from Australia and Africa (Molyneux et al., 1995; de Balogh et al., 1999). These plants are reported to produce neurological disorders in livestock with clinical symptoms similar to those caused by the swainsonine-containing members of the Leguminosae. However, these species also contain the *nor*tropane alkaloids calystegines which inhibit α- and β-glucosidases and α-galactosidase and so there may be a combination of toxic effects. The calystegines are also common in the Solanaceae and there are several *Solanum* species that can poison livestock. Symptomatically, these cases resemble "locoism" (Molyneux et al., 1996). For example, *Solanum dimidiatum* causes "Crazy Cow Syndrome" in Texas (Menzies et al., 1979) and *Solanum kwebense* causes "Maldronksiekte" in South Africa (Pienaar et al., 1976). Both disorders are characterised by neurological signs of cerebellar dysfunction, including staggering and incoordination, with severe cellular vacuolation and degeneration of Purkinje cells in the brain. Both plants contain a range of calystegines, including calystegine B_2 (Nash et al., 1993). Therefore, it seems likely that these syndromes are also lysosomal storage disorders caused by glycosidase inhibition produced by the calystegines (Nash et al., 1993).

5. Therapeutic potential of polyhydroxylated alkaloids

Very few polyhydroxylated alkaloids are available commercially. Those which are available (principally DNJ, DMJ, castanospermine and swainsonine) have become standard reagents used to investigate the

potential therapeutic and biochemical applications of this class of glycosidase inhibitor. Therefore, most of the following discussion of the therapeutic applications of polyhydroxylated alkaloids is based on a limited number of compounds that have been tested thoroughly, simply because these are the only ones which are readily available. It should also be noted that the doses required for beneficial effects in human disease states are generally below those causing the toxicities described in Section 4.

5.1. Anti-cancer agents

Both catabolic and glycoprotein processing glycosidases are involved in the transformation of normal cells to cancer cells and in tumour cell invasion and migration. Many tumour cells display aberrant glycosylation due to an altered expression of glycosyltransferases (Hakomori, 1985) and it has been known for a long time that the levels of glycosidases are elevated in the sera of many patients with different tumours (Woollen and Turner, 1965). Secreted glycosidases may be involved in the degradation of the extracellular matrix in tumour cell invasion (Bernacki et al., 1985). Furthermore, the lysosomal system is highly active in transformed cells, presumably reflecting enhanced turnover of glycoproteins and other macromolecules, and possibly increased exocytosis of lysosomal hydrolases (Olden et al., 1991). The use of polyhydroxylated alkaloids to prevent the formation of aberrant asparagine-linked oligosaccharides during glycoprotein processing and to inhibit catabolic glycosidases is being actively pursued as a therapeutic strategy for cancer.

Although a number of the polyhydroxylated alkaloids have been reported to show anti-cancer activity, research has concentrated on developing swainsonine as a drug candidate for the management of human malignancies. Swainsonine has a complex mode of action in the whole animal. It inhibits the growth of tumour cells and prevents the dissemination of malignant cells from the primary tumour to secondary sites (a process known as metastasis). However, swainsonine also has a direct stimulatory effect on the immune system. There is a wealth of literature available on the anti-cancer properties of swainsonine, probing various aspects of its activity using a multitude of different rodent and human cell lines and animal models. A summary of the essential findings from this work follows.

The action of swainsonine to prevent tumour cell proliferation is related to its inhibition of the processing of their asparagine-linked oligosaccharides. Neoplastic transformation in both human and rodent tumour cells is often accompanied by increased expression of β-1,6-branched oligosaccharides in complex-type *N*-linked glycans, which are apparently required for efficient tumour cell metastasis in vivo (Dennis et al., 1990; Baptista et al., 1994). Swainsonine blocks expression of complex-type oligosaccharides in malignant cells. It would seem that loss or truncation of β-1,6-branched oligosaccharides in metastatic tumour cells has the effect of reducing cell motility by increasing cellular adhesion, it reduces their capacity to invade other tissues and it also reduces solid tumour growth, possibly by decreasing the cellular response to autocrine growth stimulation (Dennis et al., 1990; Olden et al., 1991). However, since all cellular glycoproteins which normally express complex-type oligosaccharides are affected by swainsonine treatment, investigations were carried out to determine whether the effects of swainsonine on cellular proliferation could also be widespread amongst non-transformed tissues in the body. These studies showed that the effects of this compound would appear to be cell-specific, since it does not affect the processing of all glycoproteins equally (Olden et al., 1991; Spearman et al., 1991; Korczak and Dennis, 1993).

There is considerable evidence that swainsonine enhances the natural anti-tumour defences of the body (Kino et al., 1985; Humphries et al., 1988). In animal models, it was observed that the reduction in metastasis of tumour cells induced by swainsonine administration continued for a number of days after the drug was withdrawn (Olden et al., 1989). It was discovered that this results from the ability of swainsonine to activate immune effector cells such as natural killer cells (which are peripheral blood lymphocytes), T-lymphocytes and macrophages (White et al., 1991; Das et al., 1995). These phagocytic cells have diverse functions that include antigen presentation, cytokine production (e.g. interleukins), immune surveillance and cytocidal activity against tumour cells. However, many of the properties of these cells are apparent only after they have been activated (Das et al., 1995). Swainsonine stimulates proliferation of spleen cells, thereby increasing the numbers of natural killer cells. It also increases lymphocyte sensitivity to interleukin-2 (IL-2) and other cytokines (Yagita and Saksela, 1990; Colombo et al., 1992). Studies showed that the extent of activation by swainsonine of peritoneal macrophages to cytotoxicity against tumour cells was comparable to that obtained with known macrophage-activating agents such as interferon and bacterial lipopolysaccharide. It was associated with increased secretion of interleukin-1 (IL-1) by the macrophages, induction of protein kinase C activity and enhanced secretion of the major histocompatibility antigen on the cell surfaces (Grzegorzewski et al., 1989). Swainsonine can also induce tumouricidal activity in resident tissue-specific macrophages of both the lung and spleen (Das et al., 1995), with activation being both time- and dose-dependent. This is relevant to the clinical management of metastatic diseases since visceral organs are common sites for metastasis formation.

Before swainsonine could be used clinically, the possible adverse effects of this agent had to be evaluated.

As previously discussed, the systemic administration of high doses of swainsonine to sheep has been reported to induce a neural lysosomal mannoside storage disease (Huxtable and Dorling, 1982). However, no evidence of an overt toxic reaction was observed when swainsonine was administered orally to rodents (Huxtable and Dorling 1985) so it was considered that the mannosidosis induced by swainsonine could be a species- or tissue-specific phenomenon (Tulsiani and Touster, 1987). Swainsonine is both water and lipid soluble and therefore, it diffuses efficiently into tissues. In tissue culture swainsonine has been shown to attain concentrations inside cells similar to that in the culture medium within minutes (Chotai et al., 1983). In 1989, Mohla and co-workers compared the fates of swainsonine administered to mice by different routes (orally, intravenously, intraperitoneally and subcutaneously). They found that in all cases the compound was cleared rapidly from the blood. Bowen et al. (1993) published a preliminary pharmacokinetic evaluation of swainsonine in mice after intravenous administration. They injected one dose of 3 μg/ml which was rapidly cleared from the blood with a half-life of 31.6 min. The swainsonine rapidly entered various body organs and tissues from the blood. The highest levels were found in the bladder (owing to urinary excretion of the compound), kidney and thymus, but the lowest levels were found in the brain, which suggested that toxicity to the central nervous system may not be a problem at swainsonine levels which are sufficient to prevent metastasis. In a follow-up study (Bowen et al., 1997) swainsonine levels in various tissues were compared for up to three days after discontinuing oral administration of this compound to mice in their drinking water. These researchers found that swainsonine was predominantly retained for at least 72 h in lymphoid tissue (spleen and thymus) which was consistent with the sustained immunomodulatory and anti-metastatic properties of this compound.

The results from the tissue culture and animal models were so encouraging that the efficacy of swainsonine was examined in phase I clinical trials in humans with advanced malignancies (Baptista et al., 1994; Goss et al., 1994, 1997). In the first study, the subjects were nineteen terminally ill cancer patients with either leukemia or breast, colon, lung, pancreatic, or head and neck cancers. Swainsonine was administered by continuous intravenous infusion over 5 days in the dose range 50–550 μg/kg body weight/day. The serum concentrations of this drug reached 3–11.8 mg/l which is 100–400 times greater than the 50% inhibitory concentration for Golgi α-mannosidase II and lysosomal α-mannosidases (Baptista et al., 1994). The patients had elevated levels of the liver enzyme aspartate aminotransferase in the serum that was indicative of hepatocyte damage and they also suffered from pulmonary and peripheral oedema (Goss et al., 1994). However, the patient with head and neck cancer had over 50% tumour remission and two others showed symptomatic improvement. A second clinical trial was then undertaken by the same researchers to evaluate swainsonine by oral administration biweekly (dose range 50–600 μg/kg body weight) to 16 patients with advanced malignancies (Goss et al., 1997). Serum drug levels peaked 3–4 h following a single oral dose. The maximum tolerated dose was found to be 150 μg/kg body weight/day due to increased levels of aspartate aminotransferase in the serum and oedema, similar to that seen in the previous trial. Other adverse effects included fatigue, anorexia, abdominal pain and neurological symptoms. It was also found in both studies that the immune system was depressed rather than stimulated and it was thought that this was due to the high doses administered. Interestingly, recent observations in cases of induced chronic locoweed poisoning in cattle similarly indicate that the immune response is depressed by prolonged exposure to high doses of swainsonine (Stegelmeier et al., 1998a). Further clinical trials were suggested by Goss et al. (1997) to investigate alternative dosing schedules with lower starting doses in order to determine whether the toxic effects of swainsonine can be overcome. Indeed, swainsonine is currently undergoing phase II clinical trials in Canada with apparently very promising results (Dr. Jeremy Carver, CEO of GLYCODesign, pers. commun.).

In 1993, Dennis and co-workers synthesised derivatives of swainsonine with lipophilic groups attached to the hydroxyls at either position 2 or 8. The 2-carbonoyloxy esters of swainsonine were relatively poor inhibitors of α-mannosidases in vitro but entered cells at a rate comparable to swainsonine where they were converted to swainsonine by intracellular esterases. In vivo, the analogues were found to have comparable activities to swainsonine as stimulators of bone marrow cell proliferation. This has led to the suggestion that these or similar analogues could be useful as pro-drugs which could be preferentially hydrolysed to release swainsonine only once they were inside tumour and/or lymphoid cells. As such they could be expected to have improved pharmacological properties and reduced side-effects (Dennis et al., 1993).

In addition to swainsonine, some of the other naturally-occurring polyhydroxylated alkaloids listed in Table 1 also show various anti-cancer properties. However, none of these compounds has been exploited to nearly the same extent as swainsonine. For example, the potent α-glucosidase inhibitor castanospermine (also an indolizidine alkaloid) has been reported to inhibit experimental metastasis in mice (Ostrander et al., 1988).

Various isoenzymes of the catabolic exoglycosidase β-*N*-acetylglucosaminidase have also been investigated as potential targets for cancer therapy due to their altered expression in a range of human cancer cell types and their potential involvement in the degradation of the

extracellular matrix in tumour cell invasion (Woynarowska et al., 1992; Martino et al., 1997). β-*N*-Acetylglucosaminidases release *N*-acetyl-D-glucosamine from glycoproteins and a number of inhibitors of these enzymes have been chemically synthesised, such as 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol (Fleet et al., 1986) and a synthetic derivative of castanospermine (6-acetamido-6-deoxycastanospermine) (Liu et al., 1991). However, potent natural product inhibitors of β-*N*-acetylglucosaminidase have also been isolated from microbial sources. These include nagstatin from *Streptomyces amakusaensis*, strain MG846-fF3 (Aoyagi et al., 1992) which is a nitrogenous *N*-acetyl-D-glucosamine analogue fused with an imidazole ring. Synthetic derivatives of nagstatin have subsequently been produced with enhanced activity (Tatsuta et al., 1995). Other inhibitors of β-*N*-acetylglucosaminidase are Pyrostatins A and B (4-hydroxy-2-imino-1-methylpyrrolidine-5-carboxylic acid and 2-imino-1-methylpyrrolidine-5-carboxylic acid, respectively) which were isolated from filtrates of *Streptomyces* sp. SA-3501 (Aoyama et al., 1995).

5.2. Immune stimulants

The effect of swainsonine on bone marrow proliferation was initially investigated because its potential use in cancer therapy required that it have minimal toxicity to bone marrow and other normal tissues. In fact, a frequent limitation of the suitability of drug candidates as chemotherapeutic agents is their toxicity to normal tissues, especially bone marrow (Frei and Cannellos, 1980; Hryniuk and Levine, 1986). It was found that swainsonine stimulated cell proliferation of murine bone marrow following haematological injuries although it did not stimulate healthy bone marrow progenitor cells in vivo when administered alone (Olden et al., 1991; White et al., 1991; Klein et al., 1999). The possibility that swainsonine could confer protection against the cytotoxic effects of both cell cycle-specific and -non-specific cytotoxic anticancer agents was first examined in a murine model system by Oredipe et al. (1991). The results indicated that the intraperitoneal administration of swainsonine decreased the lethality of methotrexate, 5-fluorouracil, cyclophosphamide and doxorubicin in non-tumour-bearing mice. However, these responses were critically dependent on the dose, sequence and timing of swainsonine administration. More recently, Klein et al. (1999) reported that swainsonine protected the bone marrow cells of mice from the toxic effects of cyclophosphamide without interfering with the drug's ability to inhibit tumour growth. Also, swainsonine did not stimulate proliferation of haematopoietic tumour cells which would suggest that this drug could still be used in patients with this type of cancer.

Klein et al. (1999) also assessed the protective properties of swainsonine in vivo with the myelosuppressive agent 3′-azido-3′-deoxythymidine (known as Zidovudine or AZT), which is often used in therapy for the acquired immune deficiency syndrome (AIDS). Swainsonine administered by intraperitoneal injection increased both total bone marrow cellularity and the number of circulating white blood cells in mice treated with doses of AZT that typically lead to severe myelosuppression, which is the major dose-limiting feature in chemotherapeutic regimens for AIDS using this drug (McLeod and Hammer, 1992). In addition, swainsonine protected human myeloid progenitor cells from AZT toxicity in vitro (Klein et al., 1999).

Therefore, the possibility exists that swainsonine could be used to accelerate the recovery of bone marrow cellularity and competence following high-dose chemotherapy or autologous bone marrow transplantation, or used as an adjuvant during AZT treatment. Thus, the complications and increased risk of opportunistic infections associated with the prolonged immune-deficient state in these clinical conditions could be minimised and the cure rates of cytoreductive treatment could be significantly improved. This promising therapeutic application of swainsonine is currently being pursued in the USA in phase I and II clinical trials by the Canadian company GLYCODesign Inc., under the product code GD0039.

Finally, it is worth mentioning that the pyrroline alkaloid nectrisine (Fig. 2), which inhibits α-glucosidases in addition to α-mannosidases, has been reported to restore the immune response of immunosuppressed mice (Kayakiri et al., 1991).

5.3. Anti-diabetic agents

It can be seen from Table 2 that a large number of the naturally-occurring polyhydroxylated alkaloids are potent inhibitors of the various α-glucosidase-specific disaccharidases involved in mammalian digestion (e.g. sucrase, maltase, isomaltase, etc.). These enzymes are expressed at the surface of the epithelial cells of the brush border in the small intestine. In the late 1970s, it was realised that inhibitors of these enzymes, such as DNJ, could be used therapeutically in the oral treatment of the non-insulin-dependent (type II) diabetes mellitus (Yagi et al., 1976; Schmidt et al., 1979).

It was found that the activity of 1-deoxynojirimycin (DNJ) in vivo against intestinal sucrase was lower than that seen in vitro and this initiated a synthetic programme to produce derivatives with enhanced activity. The *N*-alkyl derivatives were most effective and this led to the development of *N*-hydroxyethyl-deoxynojirimycin (known as Miglitol or BAY m-1099) as a drug candidate (for a review, see Junge et al., 1996). This derivative has improved retention in the small intestine which increases its potential in vivo antidiabetic activity. Miglitol does not have hypoglycemic activity like insulin and the

sulfonylureas. Instead, by inhibiting the breakdown of complex carbohydrates in the gut it reduces glucose uptake and so reduces the postprandial rise in blood glucose which is characteristic of diabetes (Jacob, 1995). However, Miglitol is absorbed appreciably from the gut into the bloodstream and additional effects of this compound have been observed. It has been reported to reduce postprandial insulin secretion, lessen diabetic glucosuria and reduce the carbohydrate-driven synthesis of very low density lipoproteins (Müller, 1989). The 7-*O*-β-D-glucopyranosyl derivative of α-homonojirimycin (MDL 25637) is another drug candidate which acts in the same manner as Miglitol (Liu, 1987; Rhinehart et al., 1987). This was synthesised prior to its discovery as a natural product (Kite et al., 1990).

A naturally-occurring glycoside of DNJ (2-*O*-α-D-galactopyranosyl-deoxynojirimycin) and fagomine (which can be considered to be 2-deoxy-DNJ) have both recently been shown to have potent anti-hyperglycemic effects in isolated perfused pancreases from streptozotocin-induced diabetic mice (Kimura et al., 1995; Nojima et al., 1998). Taniguchi et al. (1998) reported that fagomine has the ability to potentiate glucose-induced insulin secretion from isolated rat pancreatic islets. Although the exact mechanism of action of this compound has not yet been elucidated, it would appear that fagomine does not affect the enzymes in the citric acid cycle, but rather it accelerates the steps in the glycolytic pathway that occur after the formation of glyceraldehyde 3-phosphate (Taniguchi et al., 1998).

Castanospermine can be regarded as a bicyclic derivative of DNJ, with an ethylene bridge between the hydroxymethyl group and the ring nitrogen. However, owing to its toxicity, this compound was considered unsuitable for therapeutic use in diabetes mellitus. Unsuccessful attempts were made to synthesise derivatives of castanospermine that were better tolerated (Junge et al., 1996).

5.4. Anti-viral agents

Another therapeutic application of polyhydroxylated alkaloids is as anti-viral agents. Inhibitors of processing α-glucosidases, such as castanospermine and DNJ, have been shown to decrease the infectivity of human immunodeficiency virus (HIV) in vitro at concentrations which are not cytotoxic to lymphocytes, whereas specific inhibitors of processing α-mannosidases (swainsonine and 1-deoxymannojirimycin) have no effect on HIV (Tyms et al., 1987; Fleet et al., 1988; Taylor et al., 1991). Castanospermine and DNJ also reduce the infectivity of other retroviruses such as the feline equivalent of HIV (Stephens et al., 1991) and human cytomegalovirus (CMV) which is an opportunistic pathogen in AIDS which is caused by HIV infection (Taylor et al., 1988).

HIV primarily infects cells of the immune system. Essential to infection is the interaction between the heavily glycosylated viral envelope glycoproteins gp120 and gp41 with the CD4 receptor which is a membrane glycoprotein found on the surface of T-lymphocytes and other cells of the immune system. In the presence of castanospermine and DNJ the glycosylation patterns of the viral coat glycoproteins are altered. Although this does not prevent formation of viral particles, they no longer have the ability to interact correctly with the CD4 receptor and so they are non-infectious (Taylor et al., 1991; Fischer et al., 1995).

Trials were conducted to try to define the structural features necessary in polyhydroxylated alkaloids for antiviral activity (Fleet et al., 1988; Karpas et al., 1988). It was discovered that *N*-substituted derivatives of DNJ were more potent in vivo than the natural product. In particular *N*-butyl-DNJ (SC-48334) had enhanced anti-HIV activity (possibly because the aliphatic chain increased uptake by the cells) and this compound was developed as a drug candidate and was evaluated in phase II clinical trials. In combination with dideoxynucleoside derivatives which target the viral reverse transcriptase activity (e.g. AZT), *N*-butyl-DNJ caused diarrhoea, abdominal pain and weight loss in human patients after oral administration (Fischl et al., 1994). Chemical modifications of *N*-butyl-DNJ were undertaken to try to eliminate the gastrointestinal toxicity. A perbutyrylated ester (SC-49483) was reported by Jacob (1995) to be undergoing a phase II clinical trial. This is effectively a pro-drug as it was shown to be absorbed from the gut and metabolised in the gastrointestinal mucosa to release the active agent *N*-butyl-DNJ into the plasma. Although the anti-viral efficacy of this compound in humans has not been reported, it apparently did not cause diarrhoea in Rhesus monkeys (Jacob, 1995).

The mechanism of action of *N*-butyl-DNJ at the molecular level appears to involve the inhibition of the host cell enzymes α-glucosidases I and II. This arrests *N*-glycan processing such that immature glucosylated *N*-glycans are present on the HIV envelope glycoproteins gp120 and gp41. Conformational changes occur in the V1 and V2 loops of gp120 when it folds in the presence of these glucosylated *N*-glycans. As a consequence, the virus can still bind to its cellular receptor CD4 but gp120 is unable to undergo the post-CD4-binding conformational change required to expose the fusion peptide of gp41 that normally facilitates entry of the virus into the host cell (Fischer et al., 1995, 1996a,b). However, because glucosidases I and II are located in the lumen of the endoplasmic reticulum, it was found that very high extracellular concentrations of *N*-butyl-DNJ were required to achieve enzyme inhibition. Karlsson et al. (1993) reported that although *N*-butyl-DNJ had a K_i of 0.2 μM against purified glucosidase I in vitro, extracellular concentrations of 0.5 mM were required to achieve inhibition of glucosidase I in intact cells in tissue

culture. When *N*-butyl-DNJ was evaluated in HIV patients, the serum levels achieved were only in the range of 10–50 μM which could explain the lower than anticipated antiviral efficacy of the drug that was observed during the clinical trials (Fischl et al., 1994; Platt and Butters, 1998). Recently, the pharmacokinetic behaviour of a more lipophilic derivative of DNJ (*N*-benzyl-DNJ) has been studied in rats (Faber et al., 1998). The plasma half life and tissue residency of this derivative were reported to be greater than those of the *N*-alkyl derivatives.

Similarly, synthetic modification of castanospermine showed that the lipophilic 6-*O*-acyl derivatives were more potent inhibitors of HIV than the natural product (Sunkara et al., 1989; Anderson et al., 1990). In particular, the 6-*O*-butanoyl derivative (MDL 28,574) was approximately twenty times more active than castanospermine and 50 times more active than *N*-butyl-DNJ (Taylor et al., 1991). However, once inside cells, 6-*O*-butanoylcastanospermine appeared to be hydrolysed to release castanospermine and hence this compound can also be considered to be a pro-drug (Bridges et al., 1995; Kang, 1996). Recently, this compound was reported to be tolerated well by patients during a phase II clinical trial (Richmond et al., 1996). Studies in vitro have shown synergistic activity against HIV type 1 and 2 replication when castanospermine (and its 6-*O*-butanoyl derivative) are combined with AZT and other similar dideoxynucleoside drugs (Johnson et al., 1989; Taylor et al., 1995).

Nectrisine (an inhibitor of both α-glucosidases and α-mannosidases) has also been reported to have anti-viral activity. It has been shown to inhibit the retrovirus Friend leukaemia virus in vivo in mice and it too can potentiate the activity of AZT (Tatatsuki et al., 1990).

5.5. Treatment of glycosphingolipid lysosomal storage diseases

The glycosphingolipid (GLS) lysosomal storage diseases result from mutations in the genes that encode the enzymes required for GLS catabolism within lysosomes. They are a relatively rare group of human disorders that can give rise to progressive neurodegeneration due to lysosomal storage within cells of the central nervous system. In the most severe forms of these diseases, death occurs in early infancy (Platt and Butters, 1998). An enzyme replacement therapy is currently available for type 1 Gaucher disease (caused by a defect in β-glucocerebrosidase) (Cox, 1994), but there are diseases associated with virtually every enzyme in the glycosphingolipid degradation pathway. However, a relatively new approach to the generic treatment of this family of disorders relies on the fact that the mutations giving rise to the enzyme defects generally do not totally eliminate the catalytic activity of the enzymes. Therefore, drugs could be used to regulate the rate of biosynthesis of the glycosphingolipids so that the amount of substrate the defective enzyme has to catabolise is reduced to a level that matches the residual enzyme activity, thus balancing synthesis with degradation and preventing storage. This type of treatment is termed "substrate deprivation".

The first step in GLS biosynthesis is the transfer of glucose from UDP-glucose to ceramide which is catalysed by the enzyme UDP-glucose: *N*-acetylsphingosine D-glucosyltransferase (EC 2.4.1.80). In 1994, Platt and co-workers discovered that this enzyme is inhibited by the α-glucosidase inhibitor *N*-butyl-DNJ (K_i 7.4μM Platt et al., 1994a). Subsequently, a galactose analogue of *N*-butyl-DNJ (*N*-butyl-deoxy-galactonojirimycin or *N*-butyl-DGJ) was found to be a more selective inhibitor of this process in vitro (Platt et al., 1994b). These compounds act as competitive inhibitors for ceramide in the glucosyltransferase assay and as non-competitive inhibitors for UDP-glucose, indicating that inhibitory activity is by ceramide mimicry. Therefore, the potential GSL lysosomal storage diseases that could be targets for treatment using *N*-butyl-DNJ and *N*-butyl-DGJ are Gaucher, Fabry, Tay-Sachs, Sandhoff, ceramide lactoside lipidosis and fucosidosis (Platt and Butters, 1998). In 1997, Platt et al. reported successfully preventing GSL storage in a mouse model of Tay-Sachs disease using *N*-butyl-DNJ.

When considering the potential clinical application of *N*-butyl-DNJ for the treatment of GLS lysosomal storage diseases, it is worth considering the findings of the clinical trials of this compound as an anti-HIV agent. In Section 5.4, it was reported that the serum levels of the compound attained after oral dosing were insufficient for inhibition of glucosidase I because the drug could not reach its target due to the location of the catalytic site of the enzyme within the lumen of the endoplasmic reticulum. However, the glucosyltransferase inhibited by *N*-butyl-DNJ is known to have its catalytic site orientated towards the cytosolic face of an early Golgi compartment which would make this enzyme target more accessible to the drug (Platt and Butters, 1995). Also, Platt and Butters (1998) considered that the serum levels reported for *N*-butyl-DNJ from the clinical trials would be sufficient to inhibit GLS biosynthesis. Since *N*-butyl-DNJ was reasonably well tolerated during the HIV clinical trials, the clinical evaluation of this compound for the treatment of GLS lysosomal storage diseases is planned (Platt and Butters, 1998).

An alternative approach involving the treatment of Fabry disease with 1-deoxy-galactonojirimycin (DGJ) itself has recently been proposed by Fan et al. (1999). Fabry disease is characterised by a deficiency in lysosomal α-galactosidase A. In some forms of the disease, this deficiency arises from a genetic mutation that affects the folding of the enzyme, although the catalytic site is unaffected. Incomplete folding is recognised by

the "quality control" system that operates in the endoplasmic reticulum resulting in abortive maturation of the mutant enzyme (Ishii et al., 1996). DGJ is a potent competitive inhibitor of α-galactosidase A. However, when lymphoblasts from Fabry patients were exposed to this compound at concentrations below those normally required for intracellular inhibition of α-galactosidase A, DGJ seemed to accelerate maturation of the mutant enzyme which was successfully transported from the endoplasmic reticulum to the Golgi apparatus and then correctly targeted to the lysosome, resulting in an elevated expression of enzyme activity. This phenomenon was also seen after oral administration of DGJ to transgenic mice that over-expressed the same mutant form of α-galactosidase A. Fan and co-workers (1999) proposed that this may be caused by DGJ occupying the catalytic site of the defective enzyme, thereby stabilising its conformation which might, in turn, control the flexibility of folding of the protein, thus allowing resumption of the glycoprotein processing and leading to maturation of the enzyme. Therefore, DGJ acts as a "chemical chaperon" to force the mutant enzyme to assume the correct conformation in order to complete its biosynthesis. Fan et al. (1999) also suggested that the binding of DGJ to α-galactosidase A could be pH-dependent such that the formation of the enzyme-inhibitor complex is favoured by the near neutral pH of the endoplasmic reticulum and dissociation occurs in the acidic environment of the lysosome. Fan and co-workers concluded that it may be possible to use other competitive inhibitors as "chemical chaperons" for the defective enzymes involved in other types of GSL lysosomal storage disorders.

5.6. Treatment of infectious agents and associated complications

One promising new area of research is the potential use of synthetic derivatives of polyhydroxylated alkaloids to specifically target the enzymes involved in the biosynthesis of the cell walls of human-pathogenic micro-organisms. For example, recent studies on the structure of mycobacterial cell walls have identified a disaccharide linker between the arabinogalactan polysaccharide and the peptidoglycan that contains L-rhamnopyranose (Besra et al., 1995). This is a common feature amongst the Actinomycetes (Lee et al., 1996). Rhamnose has no role in mammalian metabolism so compounds which interfere specifically with rhamnose metabolism should not have any deleterious effects on the host animals and this could provide a new approach to the treatment of diseases induced by mycobacteria, such as tuberculosis and leprosy, as well as diseases caused by other pathogens which also utilise rhamnose as part of their cell wall structure. The L-rhamnopyranose unit is introduced into the cell wall via the sugar nucleotide deoxythymidine diphosphorhamnose (dTDP-L-rhamnose) and it is possible that a chemotherapeutic approach to the treatment of these diseases could be to find compounds which inhibit either the microbial biosynthesis of dTDP-L-rhamnose from D-glucose-1-phosphate (a multi-enzyme conversion) (Tsukioka et al., 1997) or its subsequent incorporation into the cell wall via rhamnosyl transferases. It is reasonable to suggest that the types of structures which might inhibit these biochemical steps should contain a rhamnose fragment or a rhamnose analogue. Recently, a wide range of synthetic iminosugar derivatives have been evaluated in vitro against the L-rhamnosidase naringinase from *Penicillium decumbens* (Davis et al., 1999; Shilvock et al., 1999) and some have also been shown to inhibit the conversion of dTDP-D-glucose to dTDP-L-rhamnose in cell-free extracts of *Mycobacterium smegmatis* (Shilvock et al., 1999). Initial findings indicate the most promising candidate compounds are piperidine analogues of L-rhamnopyranose bearing amide substituents.

A similar line of investigation involves alkaloidal mimics of galactofuranose. The arabinogalactan polysaccharide in the cell walls of Actinomycetes is composed of the furanose forms of both arabinose and galactose (Lee et al., 1996). Uridine diphosphogalactofuranose (UDP-Gal*f*) is formed by the contraction of UDP-galactopyranose in a reaction catalysed by UDP-galactosyl mutase and the UDP-Gal*f* is then incorporated into the cell wall via a galactosyltransferase. Since galactofuranose has no role in mammalian metabolism, specific inhibition of either UDP-Gal mutase or the UDP-Gal*f* transferases by galactofuranose mimics may well be achieved without any harm to the mammalian hosts. In vitro studies have recently identified pyrrolidine analogues of galactofuranose that inhibit the biosynthesis of mycobacterial cell walls, probably through their action on the UDP-galactosyl mutase (Lee et al., 1997).

Gram-negative bacteria are characterised by a second membrane external to the peptidoglycan matrix. A major constituent of this membrane is lipopolysaccharide. Although lipopolysaccharides differ between bacterial species, they follow the same general format. The "anchor" is a large structure called lipid A, a polyacylated β-1,6 disaccharide of glucosamine 1,4′-bisphosphate. This is elaborated with a "core" polysaccharide and finally capped with a repeating structure called the *O*-antigen, a highly variable and species-specific saccharide (Sears and Wong, 1999). Lipopolysaccharides from many bacterial species are highly toxic to humans. They are commonly known as "endotoxins" and are responsible for septic shock. Unfortunately, treatment of pathogenic Gram-negative bacteria with antibiotics can cause the release of endotoxin from the lysed cells which can produce more acute and lethal symptoms than the original infection. A new therapeutic approach to endotoxemia is based on the generation of catalytic

antibodies capable of hydrolysing the inter-glycosidic bond in lipid A. By targeting this portion of the lipopolysaccharide it is hoped that a generic treatment can be developed for Gram-negative bacteria. Van den Berg et al. (1999) reported successfully raising polyclonal antibodies in mice that act in a manner analagous to β-*N*-acetylglucosaminidases using an immunoconjugate based on a derivative of 2-acylamido-deoxynojirimycin. This compound is a modification of the potent β-*N*-acetylglucosaminidase inhibitor 2-acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol first synthesised by Fleet et al. (1986) with the addition of an acyl chain to give a structure that more closely mimics the native lipid A. Evaluation of the efficacy of derived monoclonal antibodies is apparently on-going (van den Berg et al., 1999).

Synthetic esters of castanospermine have been shown to be useful adjuncts in the treatment of cerebral malaria (Bitonti et al., 1993). Red blood cells which are infected with the parasite *Plasmodium falciparum* become "sticky" and less deformable. This results in a build-up of cells on the vascular wall and creates occlusions to blood flow which are thought to cause the cerebral, renal and liver complications of *P. falciparum* infections. Bitonti et al. (1993) claimed that intravenous administration of various castanospermine esters could potentially reduce the adhesion of infected erythrocytes to the vascular endothelial wall.

5.7. Other therapeutic applications

Castanospermine has been investigated as a potential therapeutic agent in the treatment of autoimmune diseases such as multiple sclerosis and arthritis. Willenborg et al. (1992) found that castanospermine not only inhibited the development of arthritis in mice but it also inhibited the progression of the disease when treatment was commenced after the onset of symptoms. It prevented the passage of leucocytes through vascular subendothelial basement membranes to sites of inflammation by interfering with the biosynthesis of *N*-linked oligosaccharide hydrolases and their subsequent targeting to lysosomes. These enzymes are expressed at the surface of leucocytes and their activity is necessary for cell migration (Bartlett et al., 1995).

Castanospermine may also have a role, one day, in transplant surgery as it can inhibit the rejection of transplanted tissues. Kidney, pancreas and heart allograft survival have all been shown to be prolonged in a dose-dependent manner in the presence of castanospermine (Grochowicz et al., 1992, 1993; Hibberd et al., 1995). The compound appears to modify the expression of adhesion molecules and other cell surface glycoprotein receptors that may be involved in the alloreactive response in allograft recipients. It downregulates the expression of genes encoding the glycoproteins CD54 and CD11a, both of which are considered to have a unique and vital role in the rejection process. This downregulation may act to modulate the rejection process by influencing the interaction between endothelial cells and graft-infiltrating cells, reducing cell migration into the allograft (Grochowicz et al., 1997; Hibberd et al., 1997).

6. Concluding remarks

There is no doubt that there are many more polyhydroxylated alkaloids waiting to be discovered, including glycosides and other derivatives of those known already. Some of these, or semi-synthetic derivatives, are likely also to be inhibitors of endoglycosidases or glycosyltransferases, which opens up a wider range of potential specific applications. When more of the natural compounds become commercially available for screening there is sure to be an even wider range of potentially valuable activities found than shown to date with castanospermine, swainsonine and deoxynojirimycin.

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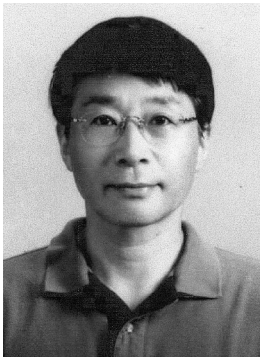
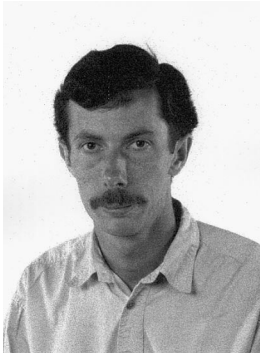
George W.J. Fleet. Having received his PhD at Cambridge University in 1969, Professor George Fleet conducted post-doctoral work with Professor Rodney Porter and Dr. Jeremy Knowles at Oxford on azide affinity labelling, and with Professor E.J. Corey at University of Harvard on prostaglandin synthesis. He is currently working at the Dyson Perrins Laboratory in Oxford. His major interests are in synthesis of carbohydrate mimetics and amino acids derived from carbohydrates likely to generate secondary structure in short sequences. He was awarded Pfizer prizes for organic synthesis in 1984 and 1985. In 1995 he received the RSC Bader Award for Organic Synthesis and was RSC Haworth Lecturer in 1999. Author of over 320 papers and published US patents.

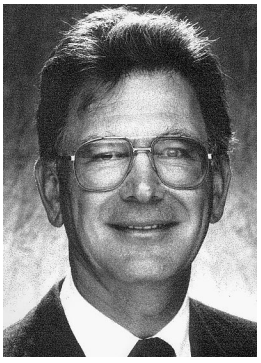
Alison A. Watson. Alison graduated in Biochemistry from King's College, London University in 1987. Her PhD research on optimising swainsonine production from the fungus *Metarhizium anisopliae* was conducted jointly at the Royal Botanic Gardens, Kew, Birkbeck College, London University, and Imperial Biotechnology Ltd at Imperial College. Post-doctoral positions at Kew Gardens involved research on various agrochemical applications of plant compounds. In 1993 she moved to the Chemistry Group at the Institute of Grassland and Environmental Research near Aberystwyth working on a variety of projects including work funded by MAFF on calystegine alkaloids in food plants and developing assays for screening compounds affecting mycobacterial cell wall biosynthesis with the Dyson Perrins Laboratory at Oxford University. Since 1999 she has been Senior Biochemist with the natural products company MolecularNature Limited. Alison has published 34 scientific papers and book chapters.



Naoki Asano. Dr. Asano received his BSc at the Science University of Tokyo in 1974. His PhD at Shizuoka College of Pharmacy led on to post-doctoral research at the Institute for Biomedical Research at the University of Texas at Austin (1986–1987). Dr. Asano is currently Associate Professor at the Faculty of Pharmaceutical Science at Hokuriku University, Japan. His research interests include searching for new structural classes of glycosidase inhibitors and discovering their therapeutic applications. Dr. Asano is author of over 80 scientific publications, book chapters and patents.



Robert J. Nash. Robert Nash graduated in Botany/Zoology (1980) at the University of Reading where Professor Jeffrey Harborne sparked his interest in the activities of natural products. A PhD at King's College London with Professor Arthur Bell on the purification and biological activity of castanospermine and related compounds led to post-doctoral research at the Royal Botanic Gardens Kew with Dr. Linda Fellows on an MRC-funded search for new anti-viral agents from plants. After 11 years at Kew, he joined the Institute of Grassland and Environmental Research (IGER) near Aberystwyth in 1993 as head of the Chemistry Group. In 1999, he became Research Director of the natural products company MolecularNature Limited based at IGER and was appointed as visiting professor of the Department of Pharmaceutical Sciences at the University of Strathclyde in January 2000. He has over 90 scientific publications, patents and book chapters.



Russell J. Molyneux. Russell Molyneux received his BSc (Chemistry) in 1960 and his PhD (Organic Chemistry) in 1963 from the University of Nottingham. Following post-doctoral and faculty appointments at the University of Oregon and Oregon State University, respectively, he joined the Western Regional Research Center (Agricultural Research Service — US Department of Agriculture) in Albany, CA as a Research Chemist, where he is currently a Project Leader. In 1993, he was awarded a Sir Frederick McMaster Fellowship (CSIRO, Australia) and in 1998 was the recipient of the Award for Advancement of Agricultural and Food Chemistry (American

Chemical Society). He was appointed as an Associate Editor of the *Journal of Agricultural and Food Chemistry* in 1999. His research interests have focused on the isolation and identification of natural products belonging to many structural classes, with particular emphasis on natural toxins. His studies of alkaloid constituents of native plants responsible for poisoning of livestock grazing rangelands in the western US have been of particular significance and have been extended to similar problems in many other parts of the world. His research on the locoweed toxin, swainsonine, led to an expansion of interest in structurally related polyhydroxy indolizidine, pyrrolizidine and tropane alkaloids from the genera *Astragalus, Oxytropis, Castanospermum* and *Ipomoea*. At present his research programme deals primarily with mycotoxins contaminating food crops and fungal metabolites responsible for phytotoxicity to grapevines in California. He is the co-editor of two books and has published 196 scientific papers, patents and book chapters.